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RESEARCH
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ADDITION IS AN
TO THE

Clinical, pathological and biochemical diversity in progressive supranuclear palsy

by

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Abstract

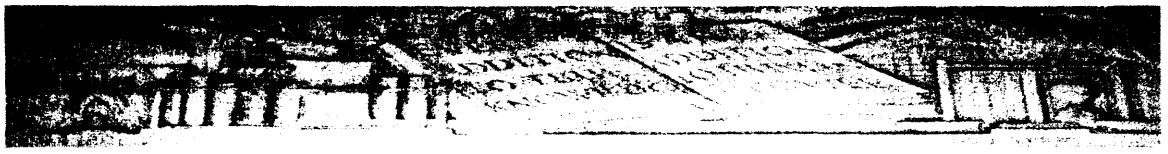
This thesis examines the clinical, pathological and biochemical diversity of progressive supranuclear palsy (PSP).

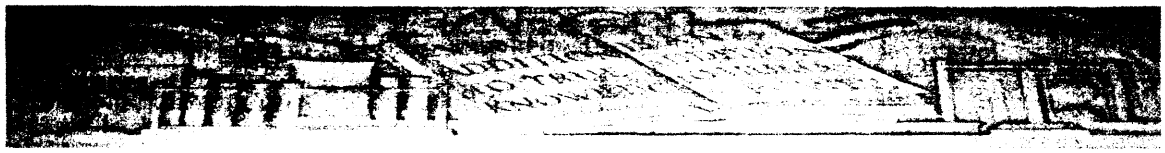
The material and patients used for these studies involved 23 clinically diagnosed living patients and 127 pathologically confirmed cases of PSP, archived at the Queen Square Brain Bank (QSBB). Differences between 'classic' PSP and 'atypical' PSP were identified, and a number of clinical features that separate them from other bradykinetic rigid syndromes were explored.

- **In addition to the clinical phenotype associated with PSP-tau pathology initially described by Richardson in 1963 (Richardson's syndrome, RS) two other distinct clinical syndromes were identified: PSP-Parkinsonism (PSP-P) and pure akinesia with gait freezing (PAGF).**
- **The following clinical features, in addition to the operational diagnostic criteria, were supportive of underlying PSP-tau pathology in patients presenting with Parkinsonism: an absence of drug induced dyskinesias, autonomic failure and visual hallucinations; the presence of falls within 6 years of disease onset; UPSIT scores above the 12th percentile for gender and age; and abnormalities in auditory startle response and auditory blink reflex.**
- **PSP-tau pathology always involved the subthalamic nucleus (STN), globus pallidus (GP) and substantia nigra (SN), but involvement outside these structures was variable and could be sub-divided into at least three different patterns.**
- **Severe tau pathology in PSP-P and PAGF was restricted to the GP, STN and SN.**
- **Co-existent pathological diagnoses did not differ between RS, PSP-P and PAGF.**
- **The ratio of pathological 4-repeat:3-repeat tau in PSP was variable. In RS the mean ratio was higher than in PSP-P (2.84 *vs.* 1.63 ($p<0.003$).**
- **Mutations of *MAPT* did not account for the diversity of clinical features**

The proposed clinical and pathological sub-classification of PSP will be helpful in clinical practice. Pathological and biochemical correlates raise the possibility that PSP-P

and PAGF may represent discrete nosological entities. Further research may ultimately lead to their absolute distinction from Richardson's syndrome and related tauopathies.





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Abbreviations

ABR	Auditory blink reflex
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APOE	Apolipoprotein E
ASR	Auditory startle response
CBS	Corticobasal syndrome
CBD	Corticobasal degeneration
CVD	Cerebrovascular disease
DLB	Dementia with Lewy bodies
EMG	Electromyographic
EOG	Electro-oculography
FTDP-17	Frontotemporal dementia with Parkinsonism linked to chromosome 17
GPe	Globus pallidus externus
GPi	Globus pallidus internus
MAPT	Microtubule associated protein tau
MMSE	Mini-mental state examination
MSA	Multiple system atrophy
NFT	Neurofibrillary tangle
NINDS	National Institute of Neurological Disorders and Stroke
NPV	Negative predictive value
OO	Orbicularis oculi
PAGF	Pure akinesia with gait freezing
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDC	Parkinsonism dementia complex of Guam
PEP	Post-encephalitic Parkinsonism
PHF	Paired helical filament
PPV	Positive predictive value
PSP	Progressive supranuclear palsy
PiD	Pick's disease
QSBB	Queen Square Brain Bank for Neurological Disorders
QSVHI	Queen Square visual hallucination inventory
Scm	Sternocleidomastoid
SKRC	Sara Koe Research Centre
SN	Substantia nigra
SPSP	Society for Progressive Supranuclear Palsy
STN	Subthalamic nucleus
UPDRS	Unified Parkinson's disease rating scale
UPSIT	University of Pennsylvania smell identification test
VH	Visual hallucinations
VP	Vascular Parkinsonism

The clinical and pathological features currently used to diagnose PSP are based on the seminal descriptions of Richardson, Steele and Olszewski. The Canadian authors predicted that with greater awareness of the condition, clinical heterogeneity would be found.

"Atypical PSP" refers to patients in whom typical PSP-tau pathology is found at autopsy, but whose clinical features have deviated from Richardson's classic description. The lack of clear-cut clinicopathological correlations has made accurate definition of these patients difficult in clinical practice.

Despite its ambiguity, a number of authors have reported pathological, biochemical and genetic differences between "atypical" patients and those whose clinical syndrome mirrored Richardson's original description.

The research undertaken for this thesis was designed to explore "atypical" PSP in detail. Specifically the aims were:

(1) to look for patterns of clinical symptoms that are present in pathologically defined PSP;

(2) to look for patterns of pathological changes in PSP and correlate them with clinical phenotypes;

(3) to examine the biochemical profiles of insoluble PSP-tau and correlate them with clinical phenotypes;

(4) to compare the tau genetic background in clinical phenotypes of PSP; and

(5) to determine the clinical features which are most helpful in identifying "atypical" PSP in life.

In the early 1960s Richardson recognised a *clinical* syndrome he designated PSP. It was unique amongst the known bradykinetic rigid syndromes and was characterised by nuchal rigidity, ophthalmoplegia, pseudobulbar palsy, dementia, and gait disturbance. His colleagues Steele and Olszewski identified pathological changes that were consistent throughout these patients, identifying what are now considered the pathological hallmarks for PSP. Because of the difficulties with confirming the clinical diagnosis of PSP, the gold standard for diagnosing PSP has become pathological. Subsequently, what was originally a clinical designation became synonymous with a pathological entity. In the decades following the original observations, improvements in pathological methodology have allowed for clearer characterisation of the neuronal and glial accumulation of insoluble tau that is seen pathologically in PSP. Advances in clinical aspects of the disease have been hampered by diagnostic criteria with low sensitivity or specificity, vagaries with atypical clinical phenotypes and the lack of a sensitive and specific clinical tool to improve diagnosis. Despite this PSP is recognised throughout the world, and appears to be the second most common cause of bradykinetic rigid syndromes. Early, accurate diagnosis is a priority for patients, but improvements in clinical differentiation from other bradykinetic rigid syndromes and tauopathies is needed to improve this.

"There is a disease which I really described in patients first seen at Sunnybrook Hospital in the late 50s and 60s that strangely enough hadn't been recognized before. It is a chronic, progressive brain disease in which they have a certain defect of eye movements, as well as a rigidity and stiffness. I gave it the name progressive supranuclear palsy (PSP). The first patient which I'd seen and realized was something new and different died and I had the brain. Shortly after Olszewski came here I said; "Now I do hope you will study this brain for me. It is a very unique and special brain of a condition that hasn't been written up before." He got John Steele who was a fellow to spend most of his year working at it and we published on this"

(J. C. Richardson, interview by the Hannah Institute for the History of Medicine Archives, University of Toronto)

Progressive supranuclear palsy

In 1955, Dr J. Clifford Richardson was visited by a good friend and 52 year old business executive because of clumsiness, trouble in seeing, and mild forgetfulness. During the next 4 years, Richardson was puzzled as his friend progressively developed an unusual constellation of signs which included supranuclear ophthalmoplegia affecting chiefly vertical gaze, pseudobulbar palsy, dysarthria, dystonic rigidity of the neck, and mild dementia.

As he was observing the evolution of this illness, Richardson serendipitously identified similar symptoms in three other middle-aged Canadian veterans who were admitted to the Neurology Service at Sunnybrook Military Hospital for long term care. The first was a West Indian labourer, who immigrated to Canada in 1913 and developed unsteady walking in 1954. The second patient was a truck driver who came from England when he was a child and who was well until his personality changed and he started to fall at the age of 49. The third veteran had come from the Ukraine in 1913 and worked as a labourer until 1956 when he developed difficulty with vision, his speech became slurred, and he had trouble in swallowing. Richardson recognised that despite different presentations, these four patients had the same hitherto unrecognised disorder.

During the next few years, Richardson came across three further patients with what also appeared to be the same condition. Though all were Canadians, they were of different ethnicities, socioeconomic status, and occupations. None had family members with neurodegenerative disease and none had a history of preceding encephalitis or toxic exposure to account for their cerebral symptoms.

By the early 1960s, five of Richardson's seven patients had died. Neuropathologists Linell and Tom at the Banting Institute examined their brains and confidently diagnosed them as having post encephalitic Parkinsonism (PEP), a post-infectious disease with neurofibrillary degeneration occurring in the wake of the encephalitis lethargica pandemic of 1915-1925. They were both quite familiar with the pathological findings of PEP and they were also aware of a case report, of a Flemish patient with identical symptoms and pathological changes that the eminent neurologist Ludo van Bogaert had also attributed to PEP. (Chavany *et al.*, 1951) However, Richardson found himself at odds with this conclusion. He pointed out that none of the

patients had a history of preceding encephalitis. Furthermore, by the 1950s PEP after von Economo's encephalitis lethargica was declining and uncommon, and most tellingly the clinical syndrome he identified was quite unlike that of classical PEP, and was unfamiliar to Canadian neurologists.

In 1962, Richardson recommended that Dr John Steele assist Professor Jerzy Olszewski, the new Professor of Neuropathology in Toronto, to thoroughly re-evaluate the pathology of his cases. At the beginning of their project in July 1962, Olszewski decided that they would each make presentations during 1963, and publish their work in 1964, in the Archives of Neurology with the order of authorship as Steele, Richardson and Olszewski.

In June 1963, at the American Neurological Association meeting in Atlantic City, Richardson presented the first clinical report of eight cases of "Heterogeneous System Degeneration" with supranuclear ophthalmoplegia, pseudobulbar palsy, nuchal dystonia and dementia. (Richardson *et al.*, 1963) He observed that this hitherto unrecognised disorder presented in the seventh and eighth decades of life and was relentlessly progressive with death occurring within nine years.

Professors Houston Merritt, Robert Schwab, and Derek Denny-Brown were the discussants. They congratulated Richardson for his detailed observations about this interesting condition which Denny-Brown thought must be quite rare since none of those attending, except McNaughton, knew of similar cases. Because there were no hereditary factors, Merritt thought it was likely that some toxic influence was responsible. The neuroepidemiologist Kurland commented on the unusual concentration of cases from Toronto and he also wondered if they could have been exposed, in common, to some regional and toxic environmental factor. In answer, Richardson observed that the group of cases reported from Toronto was a small one. The patients came from various parts of Ontario and there was nothing peculiar about their diet or exposures. One patient lived in Montreal and Van Bogaert's case, which seemed to be the same disease, came from Belgium. (Chavany *et al.*, 1951; Richardson *et al.*, 1963)

In 1959 Olszewski had taken up the position of Professor of Neuropathology at the Sunnybrook Unit, after establishing himself as pathologist and anatomist through the publication of classic atlases while at McGill University. (Klatzo, 1964) In the 12

months following Richardson's invitation to review his archived cases, Olszewski described the anatomy and histopathology of the disease and the detailed localization of the lesions in four cases. These findings were presented by Olszewski to the American Association of Neuropathologists in 1963, where he described extensive subcortical neurofibrillary degeneration in the globus pallidus, subthalamic nucleus, substantia nigra and dentate nucleus. Dr Asao Hirano, a neuropathologist at Montefiore Hospital, was present at that meeting. He had previously published pathological findings of amyotrophic lateral sclerosis (ALS) and the parkinsonism-dementia complex (PDC) occurring among Chamorros on the island of Guam, (Hirano *et al.*, 1961) and in the discussion following Olszewski's paper commented on the pathological similarities between ALS/PDC and heterogeneous system degeneration.

The following year Richardson and Olszewski, together with Steele, published their seminal paper titled "Progressive supranuclear palsy" (PSP) describing the clinical and pathological features of the disease. They argued that PSP had a distinct clinical picture despite the pathological similarities to PDC and PEP. (Steele *et al.*, 1964) The highly specific clinical picture Richardson had observed was, with considerable foresight, dependent on the similar localisation of lesions amongst their cases. Furthermore they predicted that "it is possible that further observations may broaden the clinical spectrum of the disease. In other cases, the distribution of pathological changes may be different, and thereby the clinical picture would be modified." (Steele *et al.*, 1964).

In their 1963 reports, Richardson and Olszewski had called the condition heterogeneous system degeneration, a term first used by Verhaart for a similar case he described in 1958. (Verhaart, 1958) But because they were not certain the disease was a primary degeneration or that it was related to other system degenerations, it was agreed that they should choose another term. They briefly considered "Can't look down disease", "Toronto disease", and neurosurgeon Morley suggested the eponym ROT (for Richardson, Olszewski and Tom). In the summer of 1963, Richardson proposed that it be called progressive supranuclear palsy, a clinical designation and the name by which the disease is now known, particularly in Europe. Some also refer to it as the Steele-Richardson-Olszewski syndrome, an eponym which was first used by the Montréal neurologist Andre Barbeau in 1965. (Barbeau, 1965)

In 1972, Steele completed the 1964 report by confirming PSP pathology in the two patients who were still alive at the time of the original reports. He emphasized credit for the identification of the clinical syndrome must go to Richardson. (Steele, 1972)

By the 1970s, Richardson knew that the nosological entity he had first identified as an unusual syndrome in the 1950s was a unique, universal and not uncommon neurological disease of middle and later life. Of 73 patients with PSP reported between 1951 and 1972, 51 had been male and of diverse ethnic and racial origin. With one exception (David *et al.*, 1968), no similar disorder existed in their families and their past health had usually been good. Careful enquiry seemed to exclude anything in their life habits or circumstances which might have predisposed them to the disease. In none had there been known exposure to noxious or toxic agents or any antecedent illness to suggest encephalitis. The many reports available by 1972 indicated that the disease was not restricted to geographic or climatic regions. (Steele, 1972)

Contrary to predictions of clinical and pathological heterogeneity made by Steele, Richardson and Olszewski in 1964, the 59 patients reported by others between 1964 and 1972 had, with minor exceptions, presented with similar symptoms and signs and pursued a similar course. (Steele, 1972; Steele, 1975)

Interest in bradykinetic rigid syndromes, including Parkinson's disease (PD), PEP and PSP, increased with the widespread availability of L-dopa in the 1970s. In addition to the clinical features described by Richardson, PSP was found to be characterised by poor response to L-dopa medication. (Klawans and Ringel, 1971) Recognition and differentiation of PD, Rebeiz's corticodentatonigral degeneration with neuronal achromasia (corticobasal degeneration, CBD), multiple system atrophy (Graham and Oppenheimer, 1969) and a more refined characterisation of PSP allowed for the identification of unusual cases of PSP without Richardson's classic features. Reports of pathologically diagnosed cases included presentations of pure akinesia without rigidity (Matsuo *et al.*, 1991; Verny *et al.*, 1996a), isolated dementia (Davis *et al.*, 1985; Masliah *et al.*, 1991; Josephs *et al.*, 2005), parkinsonism without dementia (Davis *et al.*, 1985; Verny *et al.*, 1996a; Birdi *et al.*, 2002) as well as a number of cases dying without recorded evidence of the distinctive supranuclear ophthalmoplegia (Pfaffenbach *et al.*, 1972; Dubas *et al.*, 1983; Davis *et al.*, 1985; Daniel *et al.*, 1995) were reported.

In 1996 diagnostic criteria were published following a meeting sponsored by the National Institute of Neurological Disorders and Stroke (NINDS) and the Society for PSP (SPSP). The NINDS-SPSP consensus statement for the diagnosis of PSP was largely based on the seminal clinical descriptions by Richardson and colleagues, (Litvan *et al.*, 2003), despite the expanding literature on, what had become loosely termed clinically "atypical PSP". The NINDS-SPSP criteria for possible PSP required the presence of a gradually progressive disorder with onset at age 40 or later, either vertical supranuclear gaze palsy or both slowing of vertical saccades and prominent postural instability with falls in the first year after onset, as well as no evidence of other diseases that could explain these features. Probable PSP required vertical supranuclear gaze palsy, prominent postural instability, and falls in the first year of onset, as well as the other features of possible PSP. Definite PSP required a history of probable or possible PSP and histopathologic evidence of typical PSP. The recognition of clinical heterogeneity in pathologically diagnosed cases of PSP has lead to the adoption of an imprecise clinical classification, which includes typical and atypical clinical subgroups. The arbitrary definitions of atypical clinical PSP have varied and are often applied retrospectively.

What is PSP?

Richardson's original designation of PSP was as a clinical entity. Despite this, in the past 3 decades "PSP" has come to be defined by the pathological changes originally described by Steele and Olszewski, probably because of the clinical heterogeneity. The diagnosis of PSP is made by the identification of tau-positive neurofibrillary tangles and neuropil threads at high density in pallidum, subthalamic nucleus, substantia nigra or pons and in lower density in other subcortical structures. In addition to neuronal tau deposits, PSP-tau also forms insoluble deposits in glial cells which are characteristically star-shaped tufted astrocytes and comma shaped coiled bodies. The pathological signature of PSP is the tufted astrocyte, which is rare in other tauopathies. (Litvan *et al.*, 1996b; Lowe *et al.*, 2002)

The original pathological observations are reflected in the current neuropathological diagnostic criteria, published in 1996. (Hauw *et al.*, 1994; Litvan *et al.*, 1996b) These criteria, allowed for an "atypical" pathological group in cases where the severity or distribution of abnormalities deviated from the original description, in particular where substantial cortical tau pathology occurred. Steele and Olszewski had reported that "no pathological evidence of frontal, cortical, or white matter involvement of consequence". (Steele *et al.*, 1964) When applied using traditional histopathological staining and sampling methods the current criteria are unable to reliably differentiate PSP from PEP. (Geddes *et al.*, 1993; Litvan *et al.*, 1996b) The differentiation between typical and atypical pathological PSP has also proved difficult because involvement of the cerebral cortex has now been widely reported in PSP, particularly the frontal and primary motor cortex. In many cases the degree of cortical involvement and pathological features overlap considerably with other diseases including PDC Guam and CBD. (Braak *et al.*, 1992; Hof *et al.*, 1992; Geddes *et al.*, 1993; Verny *et al.*, 1996a; Bergeron *et al.*, 1997; Takanashi *et al.*, 2002; Tsuboi *et al.*, 2005)

The clinical diagnosis of PSP relies on the identification of a gradually progressive disorder beginning over the age of 40 years, with early falls, a fronto-limbic dementia, a vertical supranuclear gaze paresis and axial rigidity, with poor response to L-dopa and an absence of rest tremor. The NINDS/SPSP criteria (table 1.1) incorporate

these clinical features and were published quoting a sensitivity of 83% for *possible PSP* and specificity of 100% for *probable PSP* categories. (Litvan *et al.*, 1996a) When these criteria were applied retrospectively to a different series of patients clinically and pathologically diagnosis with PSP, similar values were found (sensitivity of 85% for *possible PSP* and positive predictive value (PPV) of 84% for *probable PSP*). (Osaki *et al.*, 2004) However, when applied at the first clinic visit, early in the disease, the sensitivity was reduced to 21% for *possible PSP* and 4% for *probable PSP*. (Osaki *et al.*, 2004) These data support the clinical view that the early diagnosis of PSP can be difficult. (Daniel *et al.*, 1995; Verny *et al.*, 1996b; Litvan *et al.*, 1996c; Santacruz *et al.*, 1998; Schrag *et al.*, 1999; Nath *et al.*, 2001) Pathologically diagnosed PSP is frequently mistaken for PD, cerebrovascular disease/multi infarct state, MSA, CBD and even AD. (Jackson *et al.*, 1983; Rajput *et al.*, 1991; Hughes *et al.*, 1992a; Jellinger, 1995; Santacruz *et al.*, 1998; Lopez *et al.*, 1999)

Possible	Probable
Gradually progressive disorder	Gradually progressive disorder
Onset at age 40 or older	Onset at age 40 or older
Either vertical (upward or downward gaze) supranuclear palsy[a] or both slowing of vertical saccades and prominent postural instability with falls in the first year of the disease onset	Vertical (upward or downward gaze) supranuclear palsy[a] and prominent postural instability with falls in the first year of the disease onset
No evidence of other diseases that could explain the foregoing features, as indicated by mandatory exclusion criteria	No evidence of other diseases that could explain the foregoing features, as indicated by mandatory exclusion criteria

Table 1.1 NINDS-SPSP clinical criteria for the diagnosis of PSP (Litvan *et al.*, 1996a)

In one study of 60 clinically diagnosed cases of PSP, 13 (22%) did not have PSP-tau pathology. (Osaki *et al.*, 2004) These cases had PD dementia, PD and AD, MSA, Pick's disease (PiD), frontotemporal dementia with Parkinsonism associated with chromosome 17 (FTDP-17) and vascular disease. (Osaki *et al.*, 2004) Vascular PSP has been described in the multi-infarct state (Tanner *et al.*, 1987; Dubinsky and Jankovic, 1987; Josephs *et al.*, 2002), following hypoxic brain injury (Mokri *et al.*, 2004; Kim *et al.*, 2005), CADASIL (Van Gerpen *et al.*, 2003), primary antiphospholipid antibody syndrome (Reitblat *et al.*, 2003) and cerebral amyloid angiopathy (Dubinsky and Jankovic, 1987). Subtle clinical differences exist between many of these reported cases and pathologically proven PSP, including sudden deterioration, early memory

impairment and late falls. Other diseases which can closely resemble PSP include frontotemporal dementia with ubiquitin-only inclusions (Paviour *et al.*, 2004), FTDP-17 (Morris *et al.*, 2003), Whipple's disease (Verbuch -Heller *et al.*, 1999), striato-dentate-pallidal calcification (Watanabe *et al.*, 2003), brain tumour (Siderowf *et al.*, 1998), obstructive hydrocephalus (Curran and Lang, 1994), neurosyphilis (Murialdo *et al.*, 2000) and progressive subcortical gliosis secondary to prion disease (Will *et al.*, 1988; Revesz *et al.*, 1995). A single case report exists of a reversible, neuroleptic-induced PSP-like syndrome (Campdelacreu *et al.*, 2004). Other multisystem neurodegenerative disorders in which supranuclear ophthalmoplegia occurs include Kufor Rakeb disease (Williams *et al.*, 2005), Niemann-Pick type C (Neville *et al.*, 1973), Joseph's disease (Rosenberg *et al.*, 1976) and Lewy body pathology (Fearnley *et al.*, 1991; de Bruin *et al.*, 1992), although clinical confusion with PSP would be unusual in most of these conditions.

Early, definitive diagnosis may be beneficial on many levels in neurodegenerative disease, and is demanded by most patients, but there is little literature on the impact of timely diagnosis. In PSP the mean time to definitive diagnosis in one North American study was found to be 33.4 months in men and 24.1 months in women. (Santacruz *et al.*, 1998) Patient contributions to PSP advocacy newsletters frequently report frustration at delayed diagnosis and relief when a definitive diagnosis is finally made.(PSP (Europe) Association Newsletter) In PD "satisfaction with the explanation of the condition at diagnosis" is the second most significant predictor of health related quality of life, after depression, having significantly more impact than disease severity or medication use. (The Global Parkinson's Disease Survey (GPDS) Steering Committee, 2002)

Only with a definitive diagnosis can issues such as prognosis and planning for the future, therapeutic options and genetic counselling be appropriately dealt with. Community resource allocation and discussion of end-of-life issues also cannot be dealt with adequately without "a name for the disease". Caregivers and relatives, as well as patients, need to be able to "identify" with a specific disease. (Groves and Forrest, 2005)

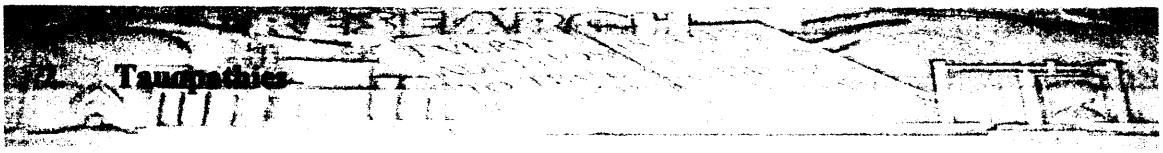
This is particularly apposite to PSP where deterioration in function can be marked in the first 12 months. (Litvan *et al.*, 1996c; Nath *et al.*, 2003) PSP, in common with other neurodegenerative bradykinetic rigid syndromes is often referred to as atypical Parkinsonism, Parkinson's-plus or simply 'bradykinetic rigid syndrome'. (Jankovic,

1989; Schrag *et al.*, 2000; Diroma *et al.*, 2003; Tolosa *et al.*, 2006) This terminology, although having the benefits of honesty where uncertainty exists, is unhelpful in providing patients and their caregiver's much insight into prognosis. Furthermore it may heighten the alienation that is common in the early stages of a chronic neurodegenerative disease. (Groves and Forrest, 2005) No support groups for "atypical Parkinsonism" exist and internet searches using these terms are more likely to yield unhelpful results.

Clinical acumen is essential for the accurate diagnosis of PSP due to the absence of biomarkers. Even when considered in the early differential diagnosis, efforts to make a definitive diagnosis are frequently impaired by the clinical heterogeneity at presentation reflected in the poor sensitivity of the most stringent diagnostic criteria. (Osaki *et al.*, 2004) Falls within the first 12 months of disease are central to the NINDS/SPSP diagnostic criteria. In the data used to prepare these criteria, the mean time to first fall was 6 months, but the range exceeded 150 months, making "late" falls the single biggest factor limiting the sensitivity of the criteria. (Wenning *et al.*, 1999) Vertical supranuclear gaze palsy is also a prerequisite for the diagnosis, but some patients develop it many years after disease onset and occasionally never. (Imai *et al.*, 1987; Daniel *et al.*, 1995; Litvan *et al.*, 1996a; Birdi *et al.*, 2002) An absence of improvement with L-dopa treatment is one of the supportive criteria for the diagnosis of PSP, however, even in the NINDS/SPSP cohort 20% of patients had some initial response, and in other reports up to a third of patients with pathologically diagnosed PSP can improve with L-dopa. (Verny *et al.*, 1996b; Litvan *et al.*, 1997; Birdi *et al.*, 2002)

Richardson was surprised that the clinical syndrome he reported from Toronto had not been reported earlier and in 1963 suggested that "a good many cases of the same disease will be identified in other areas". (Richardson *et al.*, 1963) True estimates of PSP prevalence are difficult as they are affected by the same issues that limit accurate, early diagnosis. In addition, methods of case finding and population size can result in 20-fold variations in crude prevalence rate estimates. (Nath *et al.*, 2001) The best studies of prevalence are community-based, derived from primary care, utilising sensitive inclusion criteria, personal examination and validated diagnostic criteria. Two studies in the UK that satisfy these requirements report a crude prevalence rate of 6.5 per 100,000 and an age adjusted prevalence of 5.0 per 100,000. (Schrag *et al.*, 1999; Nath *et al.*, 2001)

Another study using similar methodology estimated crude and age adjusted prevalence in Yonago, Japan to be 5.8 and 5.0 per 100,000 respectively. (Kawashima *et al.*, 2004) In Northwest Italy and the Faroe Islands, in studies designed primarily to determine the prevalence of PD, the prevalence of PSP has been estimated as 3.2 and 4.6 per 100,000 respectively. Overall these rates are similar to prevalence rates reported in motor neurone disease, where disease duration is shorter, (Roman, 1996) and more than 25 times less than rates in PD, where disease duration is almost twice that in PSP. (Porter *et al.*, 2006)



The pathological distinction of PSP from other neurodegenerative diseases characterised by accumulation of tau protein is not as clear cut as originally argued. Tauopathies encompass more than 20 clinicopathological entities including PSP, Alzheimer's disease, the most common tauopathy, Pick's disease, CBD, PEP and PDC-Guam. Important clinical, pathological, biochemical and genetic similarities exist within this spectrum of diseases and have helped to advance our understanding of the aetiological factors that initiate neurodegeneration and tau accumulation. This has enhanced the need for identification of the genetic and biochemical characteristics of PSP.

Introduction

Aggregation of proteins is now recognised as a salient feature of a large number of neurodegenerative disease (disorders of protein misfolding). It is now possible to classify neurodegenerative diseases not only by their clinical characteristics and histological features, but also by the characteristics of the proteins that accumulate in neuronal, glial and muscle cells. The microtubule associated protein *tau* is ubiquitous in the adult brain. Its normal function is to promote microtubule assembly from tubulin subunits and also to stabilise microtubules, which are important structural building blocks and transporters in neuronal cells. Normal tau protein binds to microtubules in axons, but in certain neurodegenerative diseases it is redistributed to the cell body where it accumulates to form insoluble, fibrillary deposits. (Goedert, 2004) The group of diseases characterised by accumulation of tau resulting in formation of neurofibrillary tangles and, in some, also glial filamentous inclusions, are collectively known as tauopathies.

Neurofibrillary tangles were recognised as pathological features of neurodegeneration by Alzheimer in 1907. However, it was not until the 1960s that the composition of these insoluble proteinaceous deposits began to be unravelled, when paired helical filaments were identified by electron microscopy. (Kidd, 1963) Two decades later the identification of tau protein, as the major component of these filaments, allowed for immunohistochemical recognition of tau in a number of other neurodegenerative conditions. Its importance in the aetiopathogenesis of

neurodegeneration was firmly established by the discovery of a number of families with autosomal dominant frontotemporal dementia and Parkinsonism with mutations in the tau gene and tau-positive inclusions in neuronal and in several variants of the disease, also glial cells (Hutton *et al.*, 1998). In other tauopathies the molecular events that initiate conformational changes in normal tau protein leading to aggregated protein, filament formation, neuronal dysfunction and finally cell death are largely unknown.

More than 20 different degenerative disorders are known at present to be characterised by neurofibrillary tangle formation.(Table 2.1) Most cause dementia or degeneration of the motor system, including Alzheimer's disease, the most common of the tauopathies, PSP, CBD, FTDP-17, PiD, PEP, dementia pugilistica, Down's syndrome, PDC Guam and argyrophilic grain disease. The presence of insoluble tau inclusions and neuronal loss in all of these diseases implies a common mechanism involved in cell injury and death.

Tauopathies can be divided according to coexistent pathological features. (Table 2.1) In primary tauopathies tau accumulation is the only finding that accompanies the neuronal loss and gliosis. In secondary tauopathies such as AD, the pathological accumulation of insoluble tau occurs in combination with another pathological process, such as amyloid deposition.

Molecular biology

Tau protein is the most abundant microtubule-associated protein in the brain. It was designated the Greek letter T (τ, tau) for its ability to induce tubule formation through assembly of tubulin. (Weingarten *et al.*, 1975) In the adult human brain it exists as six isoforms, differing by the presence or absence of 29- or 59-amino acid inserts located in the amino terminal end and a 31-amino acid repeat in the microtubule-binding domain. The isoforms can be further sub-divided depending on whether this microtubule binding domain harbours 3-repeat or 4-repeat sequences. In normal adult brain there are similar levels of 3-repeat tau (3R) and 4-repeat tau (4R), but in neurodegenerative disorders this ratio is altered.

The gene encoding tau is on the long arm of chromosome 17, and is made up of 13 exons. (Figure 2.1) Exon 10 codes for the alternatively spliced amino acid repeat

sequence in the binding domain, and mutations here most often cause disease by altering the ratio of 3R and 4R tau. (Hutton *et al.*, 1998) Some of the identified mutations reduce the ability of tau to interact with microtubules (Hasegawa *et al.*, 1998) while others promote the assembly of tau into filaments associated with disease. (Nacharaju *et al.*, 1999) A number of commonly occurring haplotypes of the tau gene are associated with other tauopathies, and although the precise mechanisms are yet to be determined the central importance of tau for these diseases is beyond doubt. (Pittman *et al.*, 2005)

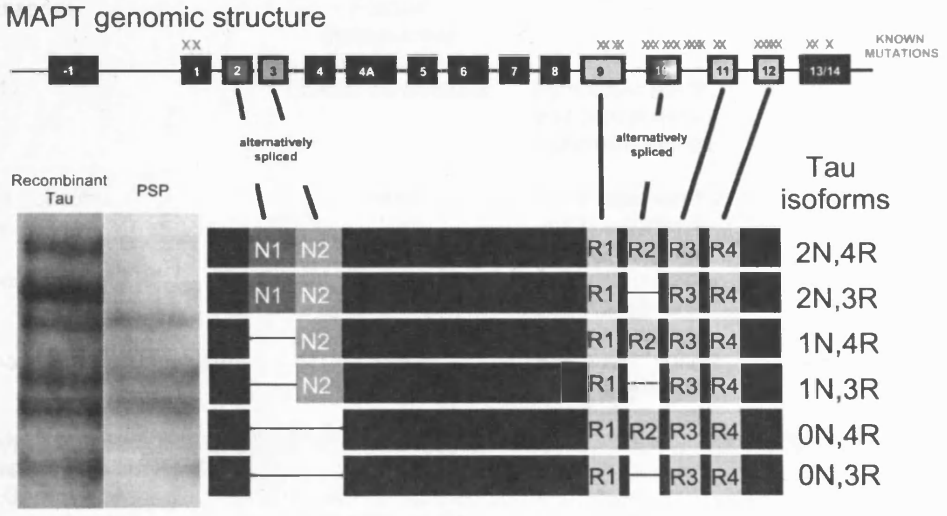


Figure 2.1 Microtubule associated protein tau, and six tau isoforms produced by alternatively spliced exons 2, 3 and 10 with western blot of recombinant tau and insoluble tau from PSP

	Pattern of dementia	Movement disorder	L-dopa response	3R:4R	Associated genes
Primary tauopathies					
Progressive supranuclear palsy					
Richardson's syndrome	Frontal dysexecutive	Axial rigidity, postural instability, bradykinesia, ophthalmoplegia	-/+	1:2-4	MAPT H1
PSP-P	Late frontal dysexecutive	Asymmetric onset, axial rigidity, tremor, late falls	++	1:1-2	MAPT H1
Argyrophilic grain disease	Limbic dementia	No	-	1:2	MAPT H1
Corticobasal degeneration	Parietal, frontal dysexecutive	Asymmetric Parkinsonism alien limb	-	1:2	MAPT H1
Pick's disease	Frontal dysexecutive PNFA, SD	Rare	-	3:1	-
FTDP-17	Frontal behavioural	Symmetric rigidity and bradykinesia, ophthalmoplegia	-/+	1:2 1:1 2:1	Multiple mutations/ deletions of *MAPT*
Post encephalitic Parkinsonism	Rare	Symmetric rigidity and bradykinesia, ophthalmoplegia	++	1:1	-
PDC Guam	Frontal dysexecutive, cortical	Symmetric rigidity and bradykinesia, ophthalmoplegia	-	1:1	-
Guadeloupean Parkinsonism	Frontal dysexecutive	Symmetric rigidity and bradykinesia, ophthalmoplegia	-/+	1:2	-
Secondary tauopathies					
Associated with amyloid deposition					
Alzheimer's disease	Amnestic, cortical	Rare	-	1:1	APP, PS1, PS2
Down's syndrome	Amnestic, cortical	No	-	1:1	Trisomy 21
Dementia pugilistica	Amnestic, cortical	Parkinsonism	-	1:1	ApoE4 risk factor
Familial British dementia	Amnestic, cortical	No	-		BRI2
Familial Danish Dementia					
In association with other pathology					
Myotonic dystrophy	Frontal behavioural	No	-	2:1	DMPK
Hallevorden-Spatz disease	Mental retardation	Gait disturbance, extrapyramidal syndrome	-		PANK2
Niemann Pick Type C	Mental retardation, psychosis	Dystonia, ataxia, ophthalmoplegia	-		NPC1 NPC2

Table 2.1 Most prevalent tauopathies. PNFA progressive non-fluent aphasia; SD semantic dementia; FTDP-17 frontotemporal dementia with Parkinsonism associated with chromosome 17; APP amyloid precursor protein; PS presenilin; Apo E Apolipoprotein E ε4; DMPK dystonia myotonica protein kinase; PANK pantothenate kinase; NPC Niemann Pick type C

PSP

Tau-positive neurofibrillary tangles and neuropil threads occur in high density in specific subcortical structures, and to varying degrees in the cerebral cortex. The pathological signature of PSP is the tufted astrocyte, which is rare in the other tauopathies. (Litvan *et al.*, 1996b; Lowe *et al.*, 2002)

The factors which lead to tau accumulation and selective vulnerability of the basal ganglia and upper brainstem in PSP are not clear. Insoluble tau exists predominantly as straight tubules, which have a slightly thinner diameter than the predominantly paired helical filaments of AD. (Roy *et al.*, 1974) PSP tau also forms insoluble deposits in glial cells which are characteristically star-shaped tufted astrocytes (figure 2.2) and comma shaped coiled bodies. Typically 4R tau is present in the glial and neuronal deposits, which are expressed as a doublet band pattern on western immunoblotting of phosphorylated tau. (de Silva *et al.*, 2003) Triplet patterns have also been reported in some cases, reflecting relatively more 3R tau and overlap with the triplet banding that is characteristic of AD. (Hanger *et al.*, 2002; Morris *et al.*, 2002a) Unlike AD, there is no clear pattern of pathological progression in PSP.

PSP is a sporadic disorder, but genetic susceptibility has been found in association with the H1c haplotype of the tau gene (microtubule associated protein tau, *MAPT*). (Pittman *et al.*, 2005) However, mutations of *MAPT* have been identified in patients with clinical and pathological features similar to sporadic PSP.

Corticobasal degeneration

The syndrome of CBD was first recognised as a discrete nosological entity in the late 1960s. (Rebeiz *et al.*, 1967) Subsequent clinicopathological reports have expanded the clinical phenotype and have frustrated attempts to produce consensus diagnostic criteria. The classic presentation is unilateral limb rigidity with myoclonus, dystonia and cortical sensory loss which can ultimately lead to the alien limb syndrome. The alien limb may present with abnormal limb movement with seemingly purposeful actions, levitation and an associated feeling of not owning the limb and results from parietal lobe degeneration. Other clinical presentations are common, and include non-fluent aphasia, a frontal lobe behavioural syndrome and apraxia with levodopa non-responsive

Parkinsonism. Disease onset is in the sixth or seventh decade and disease duration is less than 10 years. The disease is rare, the incidence is estimated as being less than 1 per 100,000 per year, and so is 10 times less common than PSP. (Litvan *et al.*, 2000) CBD is defined pathologically by the presence of swollen neurons, also known as achromatic or ballooned neurons or Pick cells and prominent diffuse cortical glial tau pathology, including neuropil threads, coiled bodies and astrocytic plaques. (figure 2.2)

Many of the features of pathological tau accumulation in CBD are shared with PSP. There are similarities in tau biochemistry; both accumulate predominantly 4R tau in neuronal and glial pathology, although in CBD the tau in both is made of predominantly twisted, helical filaments similar to AD. (Feany and Dickson, 1995) As well as in PSP, the tau H1c susceptibility haplotype is also strongly associated with CBD pathology. (Pittman *et al.*, 2005) CBD is differentiated from PSP pathologically by the presence of ballooned neurones, which are only weakly immunoreactive to tau, and tau-positive astrocytic plaques. In general, tau pathology in CBD favours a more cortical distribution than in PSP, though substantial overlap exists. (Feany and Dickson, 1995; Tsuboi *et al.*, 2005)

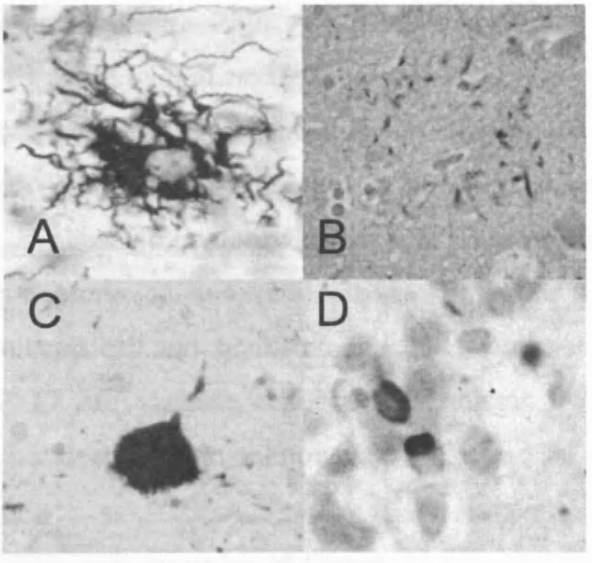


Figure 2.2 **A,** tufted astrocyte (x 40); **B,** astrocytic plaque (x 20); **C,** neurofibrillary tangle (x 40); **D** Pick bodies (x 40). AT8 anti-tau antibody.

Frontotemporal dementia with Parkinsonism associated with chromosome 17

This condition comprises a group of familial, autosomal dominant, progressive adult onset neurodegenerative syndromes in which behavioural, cognitive and motor disturbances are caused by mutations in the tau gene, of which more than 30 have now been identified in over 100 families. (Goedert and Jakes, 2005) The clinical manifestations are protean but can be predicted, to some extent, by the site of the mutation in the tau gene. Changes in personality and behaviour dominate the early clinical picture in many patients, with social disinhibition, aggressive outbursts, compulsive behaviour, gambling, hyper-religiosity and a pathological liking for sweet foods. There is progressive speech disturbance with non fluent aphasia and later deterioration to mutism in some cases. Some patients present with atypical Parkinsonism with symmetrical bradykinesia, axial and limb rigidity, supranuclear ophthalmoplegia and no response to levodopa. A diagnosis of FTDP 17 is suspected when these neurological symptoms develop between the third and fifth decade, particularly if there are early progressive speech problems or a positive family history suggestive of an autosomal dominant neurodegenerative condition. (Foster *et al.*, 1997) Refractory seizures also sometimes occur.

The pathological features of FTDP 17, including the distribution and type of tau deposits, overlap substantially with PSP, AD, CBD and PiD. A molecular genetic analysis of the gene is essential to confirm the diagnosis.

The known mutations in the tau gene result in structurally abnormal tau protein (missense mutations or deletions), an alteration in the ratio of tau isoforms (exon 10 mutations) or both. These mutations cause abnormal filament formation and accumulation of tau in neurons, or, in several variants, in neuronal and glial cells in the cerebral cortex and subcortical and brainstem nuclei. Morphology and tau isoform composition in FTDP 17 are variable and the mechanisms by which these protein alterations cause neuronal death remain unknown. (Goedert and Jakes, 2005)

Parkinsonism dementia complex of Guam

In Hirano's 1961 report of an endemic disease in the Chamorro people on the western Pacific island of Guam, he described a condition characterised by Parkinsonism

and dementia in the fifth and sixth decade of life. (Hirano *et al.*, 1961) Subsequent reports have emphasised the marked clinical heterogeneity and the wide spectrum of disease which includes a PSP-like progressive, symmetric akinetic rigid state with early gait and speech disturbance with a poor response to levodopa, frontolimbic dementia, oculomotor paresis, pigmentary retinal lesions, asymmetric apraxia with dystonia and motor neurone disease. (Steele, 2005) Reports of families support phenotypic predispositions by age: those with the youngest age of onset develop motor neurone disease; those of middle age develop a Parkinsonian syndrome closely resembling PSP; and the oldest develop dementia. A similar condition on the Japanese peninsula of Kii has been described. (Kuzuhara *et al.*, 2001) The annual incidence of Parkinsonism-dementia complex (PDC) of Guam is changing, and has been decreasing since records began in the mid 20th century. The maximum incidence was about 60 per 100,000 for men and 20 per 100,000 for women. (Steele, 2005) Most new cases now have a dementing syndrome.

Tau positive neurofibrillary tangles populate the cortex and basal ganglia structures in PDC Guam, but neuropil threads and coiled bodies are sparse compared to PSP and CBD. (Wakayama *et al.*, 1993) The neurofibrillary tangles are predominantly composed of paired helical filaments although straight filaments have also been described. (Oyanagi *et al.*, 1994) The topographical distribution of tau pathology reflects the distribution of atrophy and neuronal loss, and regional differences are found in 4R:3R tau ratios, in some cases similar to PSP (Winton *et al.*, 2006) though no clear clinicopathological correlations with the different phenotypes that have been identified. (Steele, 2005)

Exciting insights into the pathogenesis of tauopathies have been predicted from the PDC Guam geographical isolate because of its high prevalence and diminishing incidence. However, more than four decades of research have failed to uncover its aetiology. No clear genetic susceptibility has been identified. (Morris *et al.*, 2004) Current efforts to identify an environmental cause are focussed on the effects of cumulative neurotoxins in the diet or infectious agents. (Steele, 2005)

Pick's disease

In 1892 Pick described focal frontal lobar atrophy in patients with aphasia, behavioural change and dementia. (Pick, 1892) The pathological heterogeneity in frontal lobe dementias meant that it was more than a century after the initial description before the pathological hallmarks and diagnostic criteria for his eponymous disease were agreed. (McKhann *et al.*, 2001) Clinically Pick's disease (PiD) is characterised by the onset of behavioural changes and aphasia in the sixth or seventh decade that progress over 10 to 15 years. (McKhann *et al.*, 2001) Features of frontal lobe dysfunction include changes in personality and social behaviour, including apathy, prominent and distressing social disinhibition, stereotypic behaviours, alterations in food preference and loss of attention to personal hygiene and appearance as well as frontal dysexecutive features including poor planning, forethought, reasoning and organization. A progressive non-fluent aphasia characterised by prominent speech production deficits leading to mutism is the commonest language disturbance encountered. (Hodges *et al.*, 2004) Rarely PSP can present with an identical phenotype, and in some cases never develop motor or oculomotor abnormalities. (Josephs *et al.*, 2006a) Semantic dementia, defined by loss of language comprehension and visual agnosia with preserved verbal fluency may also occur. There is no reliable data on the epidemiology of PiD, but clinic based, pathological series suggest a prevalence of less than 1 per 100,000 in those in their 60s. (Rosso *et al.*, 2003) Pathologically it is characterised by sharply circumscribed and asymmetrical lobar atrophy of the frontal and temporal lobes with superficial spongiosis. The diagnosis can be made by identification of tau-positive spherical cytoplasmic inclusions known as Pick bodies (figure 2.2) throughout the frontal and temporal cortices and in particular in the hippocampus. (Bergeron *et al.*, 2003) In contrast to PSP, CBD and other forms of FTD, the substantia nigra remains relatively well pigmented.

Insoluble tau accumulation develops mainly in neuronal cells and to a lesser degree in glial cells as thorn shaped astrocytes and coiled bodies. 3R tau is the predominant isoform in PiD inclusions, but 4R tau has been seen in some cases and in a few 4R is the most abundant isoform. (Zhukareva *et al.*, 2002) The accumulated filamentous material appears as predominantly paired helical filaments on electron

microscopy, similar to AD but in some cases there are only straight filaments, similar to PSP. (Zhukareva *et al.*, 2002)

PiD is considered a sporadic disease although mutations in the tau gene have been shown to cause frontotemporal dementia associated with pathology that is indistinguishable from sporadic PiD. (Bronner *et al.*, 2005) This apparent genetic heterogeneity along with the pathological and clinical heterogeneity has caused some confusion about how to precisely define PiD. The widely accepted definition of PiD is: a sporadic 3R tauopathy, characterised by the presence of Pick bodies. Some consider PiD as nothing more than another connotation for the presence of Pick bodies and it has been suggested that the term PiD should be used only in clinical practice and dropped for pathological diagnosis where classification of 3R, 4R or mixed type of tauopathy may be more useful terms. (Bronner *et al.*, 2005)

Postencephalitic Parkinsonism

Epidemic encephalitis lethargica (EL), is an infectious or post-infectious disorder, first described fully by von Economo in Vienna after the First World War. Otherwise known as "sleepy sickness" the illness had a high mortality and one of the common late complications in the survivors was postencephalitic Parkinsonism (PEP). No aetiology for EL has been identified and the condition, at least in its epidemic form, has all but disappeared. (Reid *et al.*, 2001) Similar clinical phenotypes have been reported since and are thought to be due to autoimmunity against deep grey matter neurons. (Howard and Lees, 1987; Reid *et al.*, 2001) PEP following epidemic EL was characterised clinically by Parkinsonism usually with rest tremor and no, or very slow, disease progression, and oculogyric dystonic spasms. Mortality was high initially, but those who survived often lived to old age and in these patients response to levodopa was usually good but with early emergence of dyskinesias, fluctuations and psychiatric side effects. The diagnosis hinges on a prior history of acute EL and contemporary, sporadic cases need to be carefully compared with the seminal descriptions of the epidemic form and alternative explanations excluded. (Ravenholt and Foege, 1982)

PEP is a multisystem tauopathy characterised by widespread neuronal loss along with neuronal and glial tau accumulation and severe degradation of the dopaminergic

substantia nigra. It shares histological features with PSP, CBD and PDC Guam, but ultrastructurally the neurofibrillary tangles of PEP are similar to AD. No genetic associations have been identified in PEP.

Alzheimer's disease

AD is the most common cause of dementia accounting for between 50% and 80% of all dementia and is defined by an insidious decline in cognition from a previous higher level. (Knopman, 2003) Diagnostic criteria require that two or more cognitive domains are affected including memory impairment and at least one of language, visuospatial function, executive function (including abstract reasoning and concentration) and praxis (ability to execute skilled motor activities in the absence of weakness). (American Psychiatric Association, 2000) Early involvement of memory and the absence of early eye movement abnormalities and bradykinesia distinguish it from PSP. The prevalence increases with age, doubling with every five year increment over 65 years old. (Hy and Keller, 2000) Pathologically AD is diagnosed by semi-quantitative analysis of senile plaques composed of extracellular Aβ-amyloid peptide deposits and neurofibrillary tangles composed of intraneuronal tau deposits. (Figure 2.2) To a certain extent these changes occur in normal ageing, in the absence of dementia, so the definitive pathological diagnosis of AD requires clinical correlation.

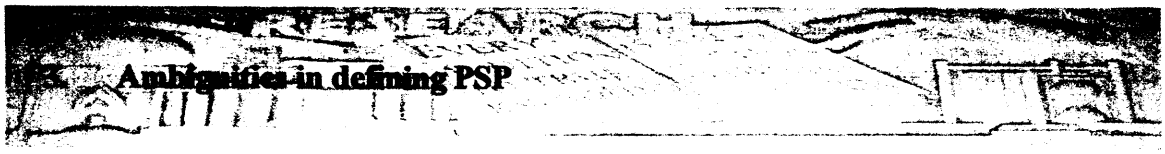
Tau accumulation in AD is likely to be a consequence of Aβ-amyloidogenic neuronal damage rather than a primary event, differentiating this process from PSP which is considered a primary tauopathy. (Gotz *et al.*, 2001) Aβ-amyloid deposition is central in the pathogenesis of AD and the balance between biogenesis and catabolism appears to be the critical factor. In addition to neuronal fibrillary tangles, insoluble tau in AD also exists as neuropil threads (derived from dendritic processes) and dystrophic neurites in senile plaques. (Braak *et al.*, 1986) Biochemical analysis of this tau reveals all six isoforms and the filaments in neurofibrillary tangles exist as paired helical filaments or twisted ribbons. (Pollanen *et al.*, 1997; Mailliot *et al.*, 1998)

Tau mutations have not been reported to cause AD. Inherited forms of AD include the highly penetrant, autosomal dominant mutations in amyloid precursor protein and presenilin 1 and 2 genes, which will lead to an increase in Aβ-amyloid deposition. In

these families tau pathology is abundant and indistinguishable from sporadic late onset AD, reflecting the importance of Aβ-amyloid deposition in the generation of tau accumulation. The apolipoprotein E ε4 allele acts as a genetic risk modifier in sporadic AD, by decreasing the age of onset of disease. (Meyer *et al.*, 1998) In patients with the ε4 allele tau load is higher than those without. (Ohm *et al.*, 1999)

Other tauopathies

The dementia associated with Down's syndrome occurs 10 to 20 years earlier than in AD, but otherwise is clinically and pathologically the same. (Wisniewski *et al.*, 1985) Dementia pugilistica, which occurs following traumatic brain injury in boxers, is also characterised by Aβ-amyloid deposition and widespread neurofibrillary tau deposition with the same isoform profile as AD. However, personality changes, cerebellar and extra-pyramidal signs are common giving it a distinct clinical picture. (Schmidt *et al.*, 2001) Argyrophilic grain disease is a newly recognised nosological entity which may account for 5% of all dementias. Pathologically it is defined as a 4R tauopathy with prominent cortical and subcortical granular changes in the neuropil and can coexist with PSP-tau pathology. (Togo *et al.*, 2002) Patients are most likely to present with memory disturbance and personality change, similar to mild Alzheimer type dementia or limbic dementia. (Tolnay *et al.*, 2002) Another geographical isolate of atypical Parkinsonism exists in the French Antilles. Clinically these patients share many features with PDC Guam, and atypical PSP, with predominantly 4R tau pathology similar to that seen in PSP. Local herbal teas made from soursop and sweetsop (annonaceae) leaves containing high levels of alkaloid neurotoxins may be responsible. (Caparros-Lefebvre and Lees, 2005) Neuronal tau deposits are also known to occur in some other genetically determined neurodegenerative conditions including Hallevorden-Spatz syndrome, Niemann Pick disease type C and myotonic dystrophy, where cognitive deterioration can occur either early or later in life and neuronal cell death occurs due to disease specific mechanisms.



"...establish first the morbid phenomena, seek then to explain them from the physiological point of view, when ever the actual state of science permits it. The inverse method, which consists of starting from anatomy and physiology in order to deduce from them the conditions of the disease, is full of dangers and beset with risks"

(J. M. Charcot. Maladies des vieillards. In: Oeuvres complètes de JM Charcot. Tome VII. Paris: Lecrosnier et Babé, 1890. [Translated into English by W.S. Tuke. Clinical lectures on senile and chronic disease by J.M. Charcot. London: The New Sydenham Society)

Description of disease in neurology has relied heavily on the anatomicoclinical method perfected by Charcot in the 19th century. He systematically categorised the clinical features of his patients and their detailed pathological findings at autopsy, revealing clinical and pathological correlations to an extent that had previously not been possible, and paving the way for a new nosological approach to nervous disease. (Goetz, 2002) This standard of disease definition was successful throughout the twentieth century and allowed for the characterisation of many neurological disorders, including PSP. (Steele *et al.*, 1964) However, the limitations of definitions that relied on strict anatomical and clinical associations became increasingly apparent, due to increasing recognition of clinical overlaps between different diseases. When these clinical conundrums were encountered in the late twentieth century, for example between PD and PSP, "the post mortem room (*became*) the temple of truth" (Calne, 2005), pathological examination *became* the source of definitive diagnosis and histological characteristics *became* the definition of disease, almost regardless of the clinical history. (Litvan *et al.*, 1996a)

With this increasing reliance on pathological findings for disease definition, the clinical description became slowly less important and consequently the name given to the disease has become less pertinent to a patient's experience. For example the current definitions of clinical PSP and pathological PSP embrace different subsets of patients.

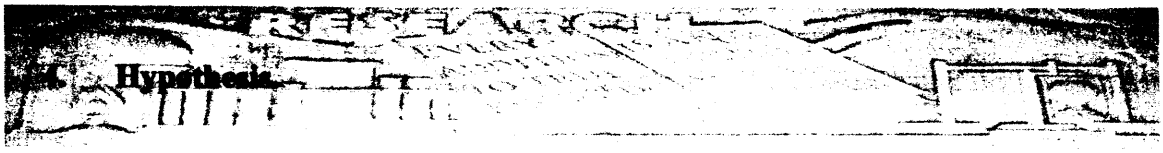
(Litvan *et al.*, 1996a; Litvan *et al.*, 1996b; Morris *et al.*, 2002a; Osaki *et al.*, 2004) and these uncertainties render the desire to provide patients with an early accurate diagnosis difficult. The current criteria are designed with clinical research in mind, and are usually unsatisfactory in providing guidelines for definitive, early diagnosis in patients who are later proven to have PSP-tau pathology. (Litvan *et al.*, 1996a; Osaki *et al.*, 2004)

The designation of PSP was originally for a clinical entity. Assuming that Richardson's definition is definitive, a number of cases with the PSP syndrome will not share the same underlying disease process. This has been estimated as being up to 25% of cases, with the alternative pathologies including Lewy body disease, MSA, frontotemporal lobar dementia with ubiquitin-only-immunoreactive neuronal changes, AD, vascular disease and prion disease. (Hughes *et al.*, 2002; Josephs and Dickson, 2003; Paviour *et al.*, 2004) To gain insight into the nature of the disease and to plan clinical trials in cases with the same underlying pathology, relying on descriptions of the clinical syndrome of PSP has substantial limitations.

The post mortem identification of PSP-tau pathology implies a certain disease experience in the patient. This assumption is not always correct and alternative clinical syndromes are consistently encountered in PSP pathological series. (Daniel *et al.*, 1995; Morris *et al.*, 2002a) In addition, substantial pathological overlap occurs with other distinct clinical syndromes, including PEP and PDC Guam, and clear boundaries to separate these diseases solely on pathological grounds do not exist. (Geddes *et al.*, 1993; Litvan *et al.*, 1996b) From the patient's perspective a pathological definition of PSP does not help define their disease and any definition that relies on post mortem features effectively excludes the patient from any benefits that come with knowledge of the diagnosis.

Contemporary definitions of PSP reflect the shift towards pathological gold-standards (Josephs and Dickson, 2003), at the expense of defining the clinical syndrome as it applies to patients in the clinic. Charcot warned that such an approach was "full of dangers and beset with risks" suggesting that neglecting "morbid phenomena" in favour of anatomy and physiology ignores the essential characteristics of disease. (Charcot, 1890) From the patient's perspective, disease definition should reflect the accepted wisdom about a clinical entity, leading to interaction with their doctor relating to

prognosis, disease course, therapeutics, research, and end-of-life issues. The nature of the underlying pathology is of less importance. To achieve a more advantageous perspective of PSP for science and patients, the diagnostic emphasis needs to revert to clinical features. To enable this, clearer definitions of the clinical entity described first by Richardson, are needed and additional clinical syndromes need to be dissected out from what has been referred to in the literature as "atypical PSP". Even with the future identification of definitive biomarkers that accurately predict the underlying pathology, the clinical aspects of the disease will remain central to the patient's disease experience and require careful attention.



Pathologically defined PSP encompasses more than the classic clinical syndrome originally described by Richardson. The hypothesis of this body of work is that classic and atypical forms of PSP represent different points on a spectrum of PSP-tau disease and together these clinical differences explain some of the pathological, biochemical and genetic diversity observed in PSP.

Questions and approaches

The questions raised and approaches taken to investigate the main hypothesis and issues related to PSP are summarised below:

A) What are the clinical differences between classic and atypical PSP?

i. Do patients with atypical PSP share common clinical features?

To address these questions the clinical case notes from the series of pathologically diagnosed cases of PSP, archived at the Queen Square Brain Bank for Neurological Disorders (QSBB), were examined. Data regarding the clinical features of these patients were analysed using factor analysis which identified groups of related clinical features. Patients were then retrospectively dichotomised according to clinical groups and compared. A further, PSP-specific clinical syndrome was identified from an exhaustive search through the clinical case notes of all patients archived at the QSBB, including those with pathologically diagnosed PD, MSA, CBD, AD and VP.

B) What is the diversity in regional tau pathology in PSP?

i. Do clinical features correlate with PSP-tau pathology?

ii. Do additional pathologies contribute to clinical features in PSP?

The topographical distribution of PSP-tau lesions was studied in 42 cases of pathologically diagnosed PSP. PSP-tau pathology was assessed using AT8, RD4 and RD3 anti-tau antibodies in 20 different brain regions. The severity and distribution of

these lesions was compared between groups of patients with different clinical syndromes. These cases were systematically screened for the presence of Alzheimer pathology, Lewy body pathology and vascular disease, and clinical groups were compared.

C) What is the diversity in the isoform composition of insoluble tau aggregates in PSP?

i. Do clinical features correlate with isoform composition?

Tissue homogenates from the basal pons of 69 cases of PSP archived at QSBB were prepared. The composition of the insoluble tau isolated from this region was analysed by dephosphorylation of the guanidine-solubilised deposits followed by electrophoresis. The isoform profiles were compared to those of the soluble tau and compared between clinical groups.

D) Do known genetic risk factors influence clinical features in PSP?

The H1 haplotype of the tau gene is associated with PSP and is considered a genetic risk factor. The association of the PSP-susceptibility haplotype in pathologically diagnosed PSP was examined in the whole QSBB PSP series and then in each different PSP subgroup. DNA from this series was then screened for mutations in exons 1 and 10 of *MAPT*. The association between APOE genotype and PSP was also examined.

E) What are the clinical features that separate atypical PSP from other bradykinetic rigid syndromes?

Tests of olfactory function, the presence of visual hallucinations and abnormalities in the auditory startle response have been proposed as useful clinical discriminators between PD, PSP and other bradykinetic rigid syndromes. A group of clinic patients, diagnosed with PSP, atypical Parkinsonism or PD at the National Hospital for Neurology and Neurosurgery, were tested using a standardised smell identification test, a novel visual hallucination inventory and a modified auditory startle protocol. Clinical features were also examined in patients archived at the QSBB to identify the specificity and positive predictive value of these features in pathologically diagnosed

patients. In addition the natural history of falls and visual hallucinations was established in different pathological groups.

F) What does clinical diversity in PSP mean to patients and future research?

The anatomoclinical method of disease definition relies on correlations between clinical and then pathological features. The potential benefits of these newly recognised correlations are discussed. Power calculations were performed using data from QSBB archived patients, where different PSP subgroups were compared to estimate sample sizes needed to power clinical research trials.

Clinical heterogeneity in pathologically diagnosed PSP

The cases Richardson presented to the American Neurological Association in 1963 had a remarkably consistent clinical picture. (Richardson *et al.*, 1963) Since then a number of other clinical presentations of PSP-tau pathology have been described.

In 1974 Imai described pure akinesia without response to levodopa. (Imai and Narabayashi, 1974) The cases described were distinguished by freezing of gait, writing and speech with kinésie paradoxale but without limb rigidity or tremor. At presentation these patients were cognitively intact, had no eye movement abnormalities and, in many, there was a long disease duration without the development of other Parkinsonian features. (Mizusawa *et al.*, 1993)

PSP has been reported in several patients with isolated dementia. (Davis *et al.*, 1985; Bergeron *et al.*, 1998; Mochizuki *et al.*, 2003; Josephs *et al.*, 2005) When marked cognitive changes predominate early in the disease, PSP may rarely be confused with AD. However, in PSP, specific cognitive and behavioural changes are associated with subcortical damage and corresponding frontal lobe deafferentation leading to a characteristic dysexecutive syndrome which occurs in 50-80% of patients at some stage of the illness. (Millar *et al.*, 2006) A number of patients have also been described with progressive Parkinsonism but without dementia, further adding to the clinical heterogeneity. (Davis *et al.*, 1985; Mizusawa *et al.*, 1993; Verny *et al.*, 1996a; Birdi *et al.*, 2002)

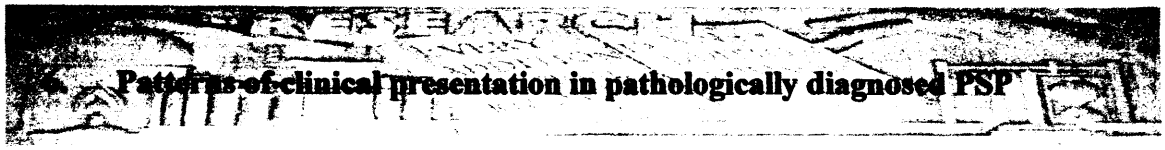
Supranuclear down gaze palsy is one of the most distinctive clinical features of PSP. Slow vertical saccades are often the first neuro-ophthalmological abnormality in PSP (Rottach *et al.*, 1996), and precede the gaze paresis. (Litvan *et al.*, 1996a) However a number of cases have been reported to have PSP pathology without ever developing a vertical supranuclear gaze palsy, or abnormalities of saccadic eye movement. (Pfaffenbach *et al.*, 1972; Dubas *et al.*, 1983; Davis *et al.*, 1985; Daniel *et al.*, 1995; Birdi *et al.*, 2002; Josephs *et al.*, 2005)

A pill rolling, 4-6 Hz rest tremor is considered to be a highly characteristic feature of PD (Gibb and Lees, 1988) and was absent in the early reports of PSP. (Richardson *et al.*, 1963; Steele *et al.*, 1964; Steele, 1972) However, rest tremor has subsequently been reported as a presenting feature in a number of patients in three different PSP pathological series. (Daniel *et al.*, 1995; Verny *et al.*, 1996a; Birdi *et al.*, 2002)

The corticobasal syndrome is characterised by asymmetric Parkinsonism, apraxia, alien limb phenomena and cortical sensory loss. (Boeve *et al.*, 1999) Although this syndrome is classically attributed to CBD, a number of pathological entities, including PSP can cause an identical phenotype. (Boeve *et al.*, 1999; Motoi *et al.*, 2004; Tsuboi *et al.*, 2005)

Attempts have been made to embrace these broader clinical features in the clinical diagnosis of PSP with typical and atypical clinical subgroups. The arbitrary definitions of these subgroups have varied and have usually been applied retrospectively. They include: the presence or absence of supranuclear gaze palsy (Daniel *et al.*, 1995; Birdi *et al.*, 2002); the presence or absence of a diagnosis of PSP in life (Morris *et al.*, 2002a; Gibb *et al.*, 2004); the application of retrospective diagnostic criteria (Morris *et al.*, 2002a); and the presence or absence of early bulbar signs or falls. (Nath *et al.*, 2003) Several authors have found genetic, prognostic or pathological differences between these clinical sub-groups. (Daniel *et al.*, 1995; Verny *et al.*, 1996a; Morris *et al.*, 2002a; Nath *et al.*, 2003)

The clinical features that have been reported to have the greatest prognostic significance in PSP are the classic clinical hallmarks of the disease described in Richardson's original report (Richardson *et al.*, 1963) and are included in the operational diagnostic criteria. (Litvan *et al.*, 1996a) Accordingly the absence of supranuclear vertical gaze palsy, early falls and early bulbar dysfunction, with a positive response to levodopa conveys a better prognosis, but patients presenting in this way are much less likely to be diagnosed as PSP, and would have been excluded by the astute eye of Richardson.



The clinical diagnosis of progressive supranuclear palsy (PSP) relies on the identification of characteristic signs and symptoms. The apparent clinical dichotomy between classic PSP and atypical PSP has been examined in this thesis. In 103 consecutive cases of pathologically confirmed PSP two clinical phenotypes have been identified by factor analysis, which have been named Richardson's syndrome (RS) and PSP-Parkinsonism (P). Cases of RS made up 54% of all cases, and were characterised by postural instability and falls, supranuclear vertical gaze palsy and cognitive dysfunction in the first two years. A second group of 33 (32%) were characterised by asymmetric onset, tremor, a moderate initial therapeutic response to levodopa and were frequently confused with Parkinson's disease (PSP-P). Fourteen cases (14%) could not be separated according to these criteria. In RS two thirds of cases were men whereas the sex distribution in PSP-P was even. Disease duration in RS was significantly shorter (5.9 vs. 9.1 years, $p<0.001$) and age at death earlier (72.1 vs. 75.5 years, $p=0.01$) than in PSP-P. A third distinct phenotype was identified in post-hoc analysis of these cases and others archived at the QSBB. A clinical syndrome of pure akinesia with gait freezing (PAGF), first described by Imai, was identified in seven cases that fulfilled proposed criteria. The mean age at onset was 61 years (range 44-78) and mean disease duration was 13 years (5-21). Akinesia of gait, speech and poverty of facial expression were common early features. Most patients were wheel chair dependent in the second half of their disease, and eye movement abnormalities typically occurred after 9 years of disease. The classic clinical description of PSP, which includes supranuclear gaze palsy, early falls and dementia does not adequately describe one third of cases in this series of pathologically confirmed cases. We propose that PSP-P and PAGF represents two discrete clinical syndromes that need to be distinguished from classical PSP (Richardson's syndrome).

6.1. Analysis of clinical features in PSP

Aims

The apparent clinical and pathological dichotomy between classic PSP and atypical PSP has led to the examination of the effect of different clinical factors on phenotype in an attempt to identify distinct subgroups that might exist alongside the clinical entity originally described by Richardson. The characteristics of these clinical subgroups were examined in an attempt to further clarify the nosology of this condition and more clearly define prognostic factors.

Materials and Methods

Patients

One hundred and three consecutive cases of pathologically diagnosed PSP, donated from all over the UK between 1988 and 2002 and stored in the Sarah Koe PSP Research Centre (SKRC) at the Institute of Neurology, UCL were reviewed. The diagnosis was made according to the NINDS-SPSP pathological criteria (Litvan *et al.*, 1996b), and was retrospectively applied to those cases acquired prior to the publication of the criteria. All patients had been assessed during life by a hospital specialist (neurologist or geriatrician).

Clinical data collection

A systematic chart review was performed on all the case notes. Specifically the comprehensive case notes of the family doctor and all of the correspondence between the family doctor and the medical specialist were reviewed. When available medical inpatient notes, inpatient consultations and emergency room admission notes were also scrutinised. A clinical data sheet was designed to record the presence or absence of clinical features either early in the disease course (within two years of first symptom onset) or at anytime during the disease. Symptoms were recorded as being *absent* if not reported, and clinical signs were recorded separately as *unknown* if they were not specifically mentioned in the notes. Where conflicting clinical features were reported the findings of the neurologist were used.

Definitions were: Age of onset: Age, in years, at the time of the first reported symptom considered to be attributable to PSP or Parkinsonism. Duration: Time between the age of onset and the age at death. Falls: The presence of any report of falls. Bradykinesia: The presence of any mention of bradykinesia or motor slowing. Cognitive decline: The presence of any perceived cognitive decline, either by the patient, patient's relative or the treating doctor. This included annotation of difficulty in concentration, reports of intellectual functional decline and comments by the family of mental slowing but did not include affective disorders. No patients underwent formal neuropsychological testing in the first twelve months of their disease. Speech disturbance: The recording of

any alteration in speech quality compared to speech prior to disease onset. Dysphagia: A record of any swallowing abnormality. Asymmetric Onset: If there was a clear difference between the signs on the left and the right asymmetry was recorded as being present. This included asymmetry of tremor, rigidity, bradykinesia or functional decline. It did not include specific tasks such as writing and using tools. Tremor: The recording of any tremor. Rigidity: The recording of axial or peripheral muscle rigidity, extra-pyramidal and pyramidal rigidity was not differentiated. Impaired Postural Reflexes: The presence of this sign was only recorded if specifically mentioned in the clinical notes. Supranuclear Gaze Palsy: The specific recording of restricted range of eye movement in the vertical plane. Impaired Saccadic or Pursuit Movements: The specific recording of abnormal saccadic or smooth pursuit eye movements. Other visual symptoms: The recording of other visual symptoms not explained by the presence of gaze palsy or impaired saccadic or pursuit movements, which evolved during the disease course. Symptoms include painful eyes, dry eyes, visual blurring, diplopia, blepharospasm and apraxia of eyelid opening. Extra axial-dystonia: The presence of dystonia in any body part apart from trunk and neck. Pyramidal signs: Pathologically brisk reflexes and/or extensor plantar response(s). Autonomic dysfunction: Either abnormal autonomic function testing or documentation of any two of: urinary urgency, frequency and nocturia without hesitancy; chronic constipation; postural hypotension; sweating abnormalities; erectile dysfunction. Dyskinesia: The presence of chorea associated with levodopa therapy. Response to levodopa: The patient and clinician's interpretation of improvement was assessed from the case notes and in some cases from the completed Parkinson's Disease Society Brain Bank (PDSBB) annual assessment forms. A self reported improvement of more than 30% coincident with the introduction of levodopa was recorded as being a positive response. This degree of response was graded by a 4 point scale modified from the PDSBB annual assessment forms: 1 - nil, or slight response (<30% improvement), 2 - moderate response (30-50% improvement), 3 - good response (51-70% improvement) and 4 - excellent response (71-100% improvement).

Statistical methods

A complete data set of clinical variables each coded as present or absent was available from 29 cases only and these were entered into a principal components analysis in order to summarise the information. Other cases had missing data on one or more variables and could not be included in this analysis. Statistical analysis was performed using SPSS for Windows (version 12.0.1). The principal components analysis was performed using clinical data from the first two years of disease. We selected the first two principal components and performed a "Varimax" rotation on these components (this rotation maximises the number of variables that have high loadings on each factor). The loadings of each variable on both of these components were plotted against each other. The plot was examined and two groups of variables in different areas of the plot were selected. A between groups, hierarchical cluster analysis using squared Euclidean distance measures was performed to check that these sets of clinical features grouped together. Those cases that exhibited a greater number of the characteristics from set 1 than from set 2 were deemed to fall within group 1, and vica versa for group 2. A cross tabulation was used to examine how many cases had a similar number of characteristics from the two variable sets. This was then applied to the cases that were excluded from the principal components analysis. Where there were missing data, we scaled up the number of positive results found (e.g. multiplied by 5/3 where status on 3 out of 5 characteristics was known). Clinical characteristics of the patients were compared between the two groups, and significance was calculated using Student's t-test for normally distributed data, and chi-square or Fisher's exact test for binary data.

Results

Clinical features

Clinical data was available for 103 pathologically confirmed cases of PSP, of which 65 were male (63%). The clinical diagnosis of PSP was made during life in 71 cases (69%), 24 (23%) were diagnosed with PD, 2 (2%) with atypical PD, 2 (2%) with MSA, 1 (1%) with CBD and in 3 a final clinical diagnosis was not established. Neurologists made the final clinical diagnosis in 87% of cases and correctly diagnosed

PSP in 72%. The mean age of onset was 66.4 years (standard deviation (SD) 12) age at death 73.5 years (SD 7.5), and disease duration was 7.0 years (SD 3.7).

The clinical features of these patients are summarised in table 6.1. There was an asymmetric onset in 28% of cases. Slowness of movement or bradykinesia was the most commonly reported feature early in the disease, occurring in 75% of cases. Falls (60%), tremor (20%), cognitive decline (29%), speech disturbance (39%), and non specific visual symptoms (21%) were early features. Rigidity and impaired postural reflexes were found in nearly half of the patients early on in the disease course. Later in the disease more clinical information was recorded. Bradykinesia was present in 98% of cases, rigidity in 98% and falls in 94%. Postural reflexes were impaired in 98% of cases, after the first two years of illness, and other signs included speech disturbance (87%), dysphagia (69%), cognitive decline (74%), tremor (23%), pyramidal signs (19%) and extra-axial dystonia (26%). Cerebellar signs (1%), autonomic dysfunction (2%), and cortical sensory loss (1%) were rare. Alien limb phenomenon was not reported.

Examination of the eye signs was not recorded in detail, beyond a statement of "cranial nerve examination normal" in more than half of the cases early in the disease. Supranuclear gaze palsy was present early in the disease in 38%, and pursuit or saccadic eye movements were abnormal in 44% of cases where the examination was recorded completely. By contrast later in the disease, 91% of these cases were recorded as having supranuclear gaze palsy though clinical examination was incompletely recorded in 21%. A trial of levodopa or dopamine agonist medications was recorded in 91 of 103 cases (88%). The trial was not recorded or was not performed in the remainder. In 32% of cases there was a better than 30% improvement in symptoms coincident with the initiation of the medication. The duration of treatment, and continued efficacy were not measured. In four cases dyskinesias were recorded (4% of those treated).

Statistical analysis

The first two components from the principal component analysis performed on data from the first two years of disease explained 42% of the variance seen in all cases. The loading of each variable on one component was plotted against its loading on the other component. (Figure 6.1) This suggested that the variables fell into two approximate

groups (characteristic set 1 with high positive loadings on component 1 and characteristic set 2 with positive loadings on component 2 and negative loadings on component 1). The variables grouped together in each characteristic set were: set 1 – falls, cognitive decline, supranuclear gaze palsy, abnormal saccadic/pursuit movements, postural instability; set 2 – tremor, bradykinesia, asymmetrical onset, extra axial dystonia, response to levodopa. A cluster analysis of clinical variables confirmed this grouping of variables as reasonable. (Figure 6.2) This suggests that sufficient information was incorporated into the first two components of variance in order to determine groupings of characteristics between patients.

The frequency of the two sets of characteristics in the 29 cases with a complete data set is summarised in table 6.2A. Fourteen cases (48%) had more features in characteristic set 1 than set 2, and were deemed to belong to group 1. Nine cases (31%) had more features in characteristic set 2, and were deemed to belong to group 2. Six cases (21%) had an equal number of features in each characteristic set.

The frequency of the two sets of characteristics in the 71 cases with incomplete data is shown in table 6.2B. Five cases (7.4%) had an equal number of features in each characteristic set. Three cases were excluded from the analysis because of insufficient clinical information. The low percentage of cases that were unclassified according to this division suggests that the identified characteristics are reasonable at distinguishing two groups. The two groups share few variables from each characteristic set, suggesting that they are clinically distinct.

The 66 cases that were not included in the principal components analysis, and were able to be separated into two groups with the cross tabulation, were compared according to the presence or absence of early clinical features (table 6.3). The co-occurrence of clinical features appeared to be similar to the cases with a complete data set, included in the principal components analysis. Early bradykinesia occurred in around three quarters of cases in both groups and did not differentiate them.

	First 2 years				Throughout disease			
Clinical Feature	Present early	Absent early	Not recorded	Present in total cohort %	Present ever	Absent ever	Never recorded	Present in total cohort %
Falls	61	40	2	60	93	8	2	94
Bradykinesia	74	25	4	75	99	0	4	98
Tremor	20	79	4	20	23	76	4	23
Cognitive decline	28	68	7	29	72	25	6	74
Non-specific visual symptoms	20	77	6	21	48	49	6	50
Rigidity	43	53	7	42	95	2	6	98
Dysphagia	6	89	8	6	64	29	10	69
Speech disturbance	38	60	5	39	83	12	8	87
Impaired postural reflexes	49	30	24	62	84	2	17	98
Supranuclear gaze palsy	19	31	53	38	75	7	21	91
Abnormal pursuit or saccades	21	27	55	44	70	6	27	92
Extra-axial dystonia	7	89	7	7	25	70	8	26
Pyramidal signs	8	88	7	8	18	79	6	19
Cerebellar signs	0	10	3	0	1	98	3	1
Autonomic dysfunction	0	100	3	0	2	97	3	2
Cortical sensory loss	0	97	4	0	1	97	4	1
Asymmetric Onset	26	67	10	28				
L-dopa response					29	62	12	32
L-dopa induced dyskinesia					4	92	7	4

Table 6.1 Clinical features in 103 pathologically confirmed cases of PSP

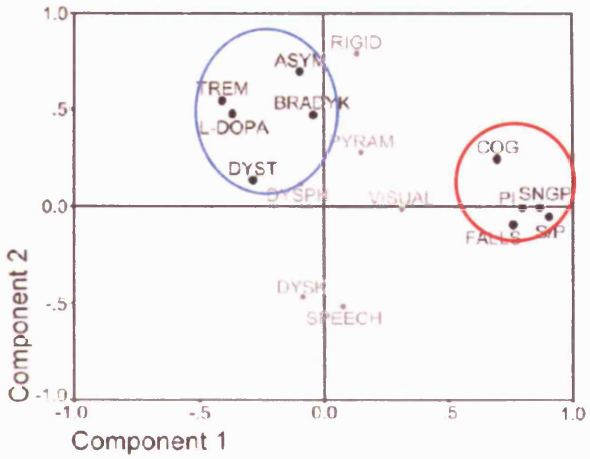


Figure 6.1 Plot of factors for components 1 and 2 derived from factor analysis. Components grouped together (red – RS; blue – PSP-P).
ASYM = asymmetric onset; TREM = tremor; L-DOPA = response to levodopa; BRADYK = bradykinesia; DYST = limb dystonia; DYSPH = dysphagia; PYRAM = pyramidal tract signs; COG = cognitive dysfunction; DYSK = dyskinesia; VISUAL = non-specific visual symptoms; PI = postural instability; SNGP = supranuclear gaze palsy; S/P = abnormal saccadic or pursuit movements; SPEECH = speech disturbance.

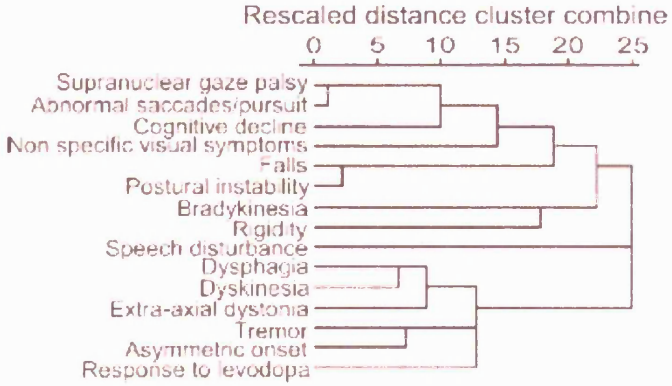


Figure 6.2 Hierarchical cluster analysis of clinical variables: dendrogram using average linkage (between groups).

2A

Set 1	Set 2 0	1	2	3	4	Total
0	1	1	1	2	2	7
1	0	2	0	0	0	2
2	1	0	3	2	0	6
3	0	0	0	0	1	1
4	2	2	1	1	0	6
5	0	6	1	0	0	7
Total	4	11	6	5	3	29

2B

Set 1	Set 2 0	1	2	3	4	Total
0	2	7	8	6	2	25
1	1	0	0	0	0	1
2	0	1	1	1	0	3
3	2	10	4	2	0	18
4	1	3	3	0	0	7
5	3	8	2	4	0	17
Total	9	29	18	13	2	71

Table 6.2 (**A**) Cross tabulation of the number of characteristics in each of two sets exhibited by each case for initial data set, and (**B**) for the second data set where at least one variable was missing
Set 1 (RS): falls, cognitive decline, supranuclear gaze palsy, abnormal saccades/pursuit movements, postural instability. Set 2 (PSP-P): tremor, bradykinesia, asymmetric onset, non-axial dystonia, response to levodopa.

	Group 1 *% reported as present*	Group 2 *% reported as present*	*p value*
Symptoms			
*Falls	85.7 (36/42)	0% (0/24)	<0.001[b]
*Cognitive decline	50.0 (19/38)	0 (0/22)	<0.001[b]
*Tremor	9.8 (4/41)	39.1 (9/23)	0.007[b]
Speech disturbance	32.5 (13/40)	26.1 (6/23)	0.406[b]
Dysphagia	2.7 (1/37)	4.3 (1/23)	0.624[b]
Other visual symptoms	23.1 (9/39)	4.3 (1/23)	0.051[b]
Clinical signs			
*Asymmetric onset	17.9 (7/39)	45.0 (9/20)	0.030[b]
*Bradykinesia	75.6 (31/42)	73.9 (17/23)	0.880[a]
Rigidity	39.5 (15/38)	52.5 (12/23)	0.333[a]
*Postural Instability	84.4 (27/32)	7.7 (1/13)	<0.001[b]
*Extra-axial dystonia	0 (0/38)	8.7 (2/21)	0.138[b]
*Supranuclear gaze palsy	70.0 (7/10)	0 (0/9)	0.002[b]
*Abnormal saccades/pursuits	63.6 (7/11)	0 (0/6)	0.017[b]
Pyramidal tract signs	7.9 (3/38)	4.3 (1/23)	0.513[b]
*Response to Levodopa ever	14.3 (5/35)	50.0 (11/22)	0.005[b]

Table 6.3 Early clinical features in cases with an incomplete data set, * variables used in initial analysis; [a] chi-square analysis; [b] Fisher's exact test
Group 1: Richardson's syndrome, Group 2: PSP-P

All 89 cases that could be separated into groups were compared according to profile (table 6.4) and the late clinical features that they displayed (table 6.5). There was a difference in gender distribution between the two groups. Men were over represented in group 1 (64.3%, 95% confidence interval (CI): 52-77%), but gender distribution was equal in group 2 (51.5%, 95% CI 33-67%). The age of disease onset was not different between the two groups. The ages at death and disease duration were significantly different: those in group 1 died at a younger age, and after shorter disease duration. Falls, cognitive decline and supranuclear gaze palsy continued to be significantly associated with group 1 later in the disease ($p<0.001$). Tremor was the only clinical feature significantly associated with group 2 later in the disease though extra-axial dystonia was also more frequent in that group. There was no significant difference in the frequency of bradykinesia, speech disturbance, postural instability, pyramidal tract signs or levodopa induced dyskinesias.

	RS	PSP-P	*p value
Gender *(male, %)*	64.3	51.5	
Age at disease onset *(years)*	66.1	66.4	0.872
Age at death *(years)*	72.1	75.5	0.041
Disease duration *(years)*	5.94	9.12	<0.001

Table 6.4 Patient profiles according to clinical group, * student's t test

	Group 1 *% reported as present*	Group 2 *% reported as present*	*p value*
Symptoms			
Falls	100 (56/56)	80.6 (25/31)	0.001[a]
Cognitive decline	90.7 (49/54)	51.6 (16/31)	<0.001[a]
Tremor	13.0 (7/54)	43.8 (14/32)	0.002[b]
Speech disturbance	90.2 (46/51)	81.3 (26/32)	0.242[a]
Dysphagia	75.5 (37/49)	56.3 (18/32)	0.070[a]
Other visual symptoms	56.6 (30/53)	34.4 (11/32)	0.047[a]
Clinical signs			
Bradykinesia	98.2 (55/56)	96.9 (31/32)	0.685[a]
Rigidity	98.1 (53/54)	96.8 (30/31)	0.687[a]
Postural Instability	100 (51/51)	96 (23/24)	0.142[a]
Extra-axial dystonia	21.2 (11/51)	42.4 (13/32)	0.054[b]
Supranuclear gaze palsy	100 (50/50)	71.4 (15/21)	<0.001[a]
Pyramidal tract signs	17.0 (9/53)	15.6 (5/32)	0.561[b]
Levodopa induced dyskinesia	1.9 (1/52)	6.3 (2/32)	0.323[b]

Table 6.5 Late clinical features in 89 cases separated into distinct groups, [a] chi-square analysis; [b] Fisher's exact test.
Group 1: Richardson's syndrome, Group 2: PSP-P

Discussion

This study confirms that two distinct clinical phenotypes exist in patients with pathologically proven PSP. One was characterised by early falls, early cognitive dysfunction, abnormalities of gaze and postural instability, and the other by asymmetric onset, tremor, early bradykinesia, non-axial dystonia and a response to levodopa medications. The clinical characteristics present in this first group were similar to those first described in PSP by Richardson (Richardson *et al.*, 1963), whereas the clinical features in the second group resembled Parkinson's disease. We propose naming the first group "Richardson's syndrome" (RS) and the second "PSP-Parkinsonism" (PSP-P). RS had a shorter duration of disease and the female to male ratio was 1:1.8, whereas the gender distribution was equal in PSP-P (see table 6.4).

This series comprised 54% RS, 32% PSP-P, 11% had equal numbers of features of both subtypes and 3% lacked sufficient clinical data to be classified. These subgroups, defined by the clinical features present in the first two years of disease, represent two separate points on a spectrum of clinical features related to PSP tau pathology. The proportions of patients presenting with RS and PSP-P in this study may not reflect the proportions of these subtypes in the community because of ascertainment bias inherent in a brain banked population. (Maraganore *et al.*, 1999) Furthermore this study is limited by its retrospective nature. Despite this, the frequency of falls (94%), bradykinesia (98%), speech disturbance (87%) and dysphagia (69%) in the whole group was consistent with data reported in other clinical and clinicopathological studies. (Maher and Lees, 1986; Verny *et al.*, 1996a; Nath *et al.*, 2003) In contrast to some studies we found a higher incidence of levodopa responsiveness (32%), extra-axial dystonia (26%) and a longer duration of disease. (Maher and Lees, 1986; Nath *et al.*, 2003) The inclusion of only pathologically proven cases of PSP partially accounts for this, and is a strength of this study, allowing us to more clearly identify atypical clinical features in cases that would have been automatically excluded from clinical reports relying on PSP clinical diagnostic criteria.

Previously, attempts have been made to define clinical subgroups in cohorts of PSP (Birdi *et al.*, 2002; Morris *et al.*, 2002a; Nath *et al.*, 2003; Gibb *et al.*, 2004) and to apply this classification in small pathologically proven series (Braak *et al.*, 1992; Daniel

et al., 1995; Verny *et al.*, 1996a; Verny *et al.*, 1996b; Bergeron *et al.*, 1997). Daniel and co-workers found that in a subgroup without supranuclear gaze palsy women were over represented, age of onset was later and duration of disease was longer when compared to the group with supranuclear gaze palsy, where men were over represented. (Daniel *et al.*, 1995) No quantitative or qualitative pathological differences were distinguished, although a subsequent study on the same material showed greater neuronal loss in the nucleus interpositus in those with a supranuclear gaze palsy. (Revesz *et al.*, 1996) Birdi and collaborators reported that patients with gait or balance difficulties at onset were less likely to improve on levodopa therapy, more likely to develop supranuclear gaze palsy and had a shorter duration of disease. (Birdi *et al.*, 2002) Morris and colleagues classified pathologically confirmed cases of PSP according to the diagnosis of PSP in life and the retrospective application of diagnostic criteria for PSP. (Morris *et al.*, 2002a) All cases in their "typical PSP" subgroup had a diagnosis of PSP in life, all retrospectively satisfied the diagnostic criteria, and such cases were more likely to have the PSP susceptibility tau haplotype (H1H1). In a series where only 60% of cases had pathological confirmation, Nath and co-workers found that the subgroup with early falls and bulbar dysfunction had a shorter survival, and those with a diagnosis of probable PSP according to the NINDS-SPSP criteria had a worse prognosis. (Nath *et al.*, 2003) The variations in definition between these studies make it difficult to compare clinical groups and even more difficult to apply clinical characteristics objectively to pathological cohorts. By applying the clinical distinctions identified in our study comparisons can be made between more homogenous clinical groups potentially enabling a better understanding of the spectrum of pathological changes in PSP.

The identification of RS and PSP-P goes some way to reconcile the differences that exist between the pathological and clinical criteria for the diagnosis of PSP. This study was designed to synthesise a large body of clinical data with no a priori assumptions. Using this data driven approach we have identified two discrete syndromes in patients who have pathologically diagnosed PSP. What this strategy could not identify were clinical syndromes with characteristics that were not included in the data collection. In addition it relies on consistent syndromes to occur in large enough numbers to impact on the principal components in the factor analysis. For example gait ignition failure was

not a clinical criterion that was extracted from the case notes, and cases where it may have been the presenting feature and other features of PSP were lacking, or developed later, were infrequent enough to have no significant impact on the factor analysis. Ascertainment bias inherent in a brain bank cohort will also inevitably skew the proportion of cases in each clinical group, and may not reflect proportions in the community. Therefore, pure akinesia may still be a third distinct clinical syndrome in PSP, but it occurs in sufficiently small numbers that it has not been a significant finding in this study. Prospective clinicopathological studies are now needed to confirm further these proposed subgroups and could potentially identify less common, but distinct clinical phenotypes.

We found no cases that exhibited any of the NINDS-SPSP mandatory exclusion criteria. (Litvan *et al.*, 1996a) However, according to our data some features that are included in the NINDS-SPSP "supportive criteria" may be misleading. Early speech disturbance was found in 39% of cases, and 32% had a modest or good response to levodopa (PDSBB grade 2 or 3), though an excellent response to levodopa (PDSBB grade 4) was not recorded. While an asymmetric onset was a feature in 28%, persistent markedly asymmetric Parkinsonism was not seen.

The core clinical features of PSP appear to be bradykinesia, rigidity and postural instability and are almost always present later in the disease. Together with the supranuclear vertical ophthalmoplegia, dementia, dysarthria and pseudobulbar palsy they form the classic features of PSP. (Steele *et al.*, 1964) When these features appear in the first two years a diagnosis of RS is most likely. We have confirmed a high frequency of non-specific visual complaints early in the course of the disease and a predominance of males in the RS subgroup. (Richardson *et al.*, 1963)

The expanded clinical phenotype now includes a syndrome that, at least in the early stages, may closely resemble idiopathic PD. The presence of the PSP-P subgroup may account for the small proportion of cases in other clinicopathological reports where an incorrect antemortem diagnosis of PD was made. (Hughes *et al.*, 2001) The features which most clearly differentiate this syndrome from RS appear to be an asymmetric onset, extra-axial dystonia, tremor and benefit from levodopa. Early bradykinesia appears to be essential for the diagnosis, but does not adequately differentiate it from RS,

especially later in the disease course. Disease duration in PSP-P is significantly longer than RS (table 6.4), and to our knowledge exceeds median survival in all clinicopathological PSP case series.

The clinical features in these two groups may give some insights into the pathological substrate for PSP. Axial rigidity, bradykinesia and postural instability are universal end points of this heterogeneous degeneration and may be related to the invariable pathological findings which form the basis of the diagnostic criteria. (Litvan *et al.*, 1996a) Though supranuclear gaze palsy is seen in the later stages of most cases (table 6.5) it appears earlier in RS. This clinical finding is specifically associated with degeneration of some cholinergic brainstem structures (Juncos *et al.*, 1991) and the nucleus raphe interpositus. (Revesz *et al.*, 1996) The current study suggests that early involvement of these areas predicts early dementia and poorer prognosis. The striatal cholinergic neurons that bear the dopamine receptors are affected in PSP either by degeneration of intrinsic striatal projection neurons or by altered regulation of D2 receptors (Ruberg *et al.*, 1985; Pierot *et al.*, 1988; Arnold *et al.*, 1994; Landwehrmeyer and Palacios, 1994; Suzuki *et al.*, 2002). The partial response to levodopa in PSP-P may imply a different pattern of neurodegeneration where these projection neurons are relatively less affected. Differences in early pathological involvement of the striatum, pallidum or subthalamus may account for the observed marked clinical differences between RS and PSP-P, which appear to be two different points on a continuous spectrum of PSP tau pathology.

6.2. Post-hoc analysis of QSBB database – pure akinesia with gait freezing

Background

In 1974 Imai described what he considered to be a hitherto unrecognised clinical syndrome which he termed pure akinesia without response to levodopa. (Imai and Narabayashi, 1974) These cases were characterised by freezing or blocking during walking, writing and speaking with 'kinésie paradoxale'. Limb rigidity and rest tremor were conspicuous by their absence. The patients were also reported to have normal eye movements and no cognitive deficits. (Mizusawa *et al.*, 1993) They could be distinguished further from Parkinson's disease (PD), by the near total lack of response to levodopa. (Barbeau, 1972) Phenomenologically Imai's pure akinesia was similar to 'trepidant abasia' described by Petrén in the pre-levodopa era. (Petren, 1901) Since Imai's description a number of similar reports have occurred describing the same clinical picture under different rubrics such as Petrén's gait (Baezner and Hennerici, 2005), (isolated) gait ignition failure (Atchison *et al.*, 1993) and primary progressive gait freezing. (Achiron *et al.*, 1993) The prominence of the distinctive early gait disturbance has been emphasised in some of the more recent reports while the absence of other Parkinsonian features and dementia has been relatively overlooked. This diversity in the clinical reports has confounded clinicopathological correlation. (Quinn *et al.*, 1989; Imai and Narabayashi, 1990)

Petrén considered that diffuse arteriosclerosis was responsible for the gait disturbance in his pathologically examined patients. (Petren, 1901) This conclusion had some merit given the contemporaneous clinical and pathological associations of 'demarche à petits pas' with thrombotic encephalomalacia by Brissaud. (Brissaud, 1895) More recently it has been suggested that patients with "pure akinesia" or "primary progressive gait freezing" may have an over accumulation of hyperphosphorylated tau in subcortical structures because of the associated late development of ophthalmoplegia, nuchal rigidity and dysphagia in some cases. (Imai *et al.*, 1993; Riley *et al.*, 1994; Factor *et al.*, 2002; Factor *et al.*, 2006) This has been supported in the handful of published autopsy cases where pathology compatible with PSP has been the commonest finding.

(Homma *et al.*, 1987; Matsuo *et al.*, 1991; Mizusawa *et al.*, 1993; Yoshikawa *et al.*, 1997; Katayama *et al.*, 1998; Konishi *et al.*, 2005; Factor *et al.*, 2006) Two published cases have been diagnosed as pallidonigro-luysian atrophy (PNLA) (Konishi *et al.*, 2005; Factor *et al.*, 2006) a rare pathological diagnosis characterised by severe neuronal loss and gliosis in the globus pallidus, subthalamic nucleus and substantia nigra. (Contamin *et al.*, 1971; Kawai *et al.*, 1993) The neuropathological characteristics of PNLA have not been fully delineated but there is evidence that this condition substantially overlaps with PSP-tau pathology. (Mori *et al.*, 2001)

Imai's pure akinesia is distinct from both RS and PSP-P and has been suggested to represent a further separate clinical phenotype. (Imai *et al.*, 1993)

Aims

The present study was undertaken to determine to what extent this clinical syndrome is represented in the Queen Square Brain Bank (QSBB) and whether it is specific for PSP-type tau pathology. Diagnostic criteria and a reclassification of the nomenclature is proposed to incorporate previous observations with the current findings, under the label of "pure akinesia with gait freezing".

Patients and Methods

A retrospective analysis was performed using the clinical case-notes from 886 cases of pathologically diagnosed neurodegenerative disease archived at the QSBB. From this 749 had sufficient clinical information and a definitive primary diagnosis for inclusion in the study (Lewy body Parkinsonism (LBP), n = 470; PSP, n = 125; multiple system atrophy (MSA), n = 84; vascular Parkinsonism (VP), n = 25; Alzheimer's disease, n = 9; corticobasal degeneration (CBD), n = 9; post-encephalitic Parkinsonism (PEP), n=5; essential tremor (ET), n=5; Huntington's disease(HD), n=5; other, n=12). The cases met the widely accepted pathological criteria for the diagnosis of these conditions. (Kosaka, 1990; Mirra *et al.*, 1991; McKeith *et al.*, 1996; Litvan *et al.*, 1996b; Gilman *et al.*, 1999; Lantos, 2000)

Data Collection

A systematic chart review was performed as described in chapter 6.1. Patients with pure akinesia were selected according to criteria, modified from Imai (Imai *et al.*, 1993). (Table 6.6) In the patients who satisfied these criteria, clinical features from disease onset to death were recorded. Where any clinical feature was present in more than half of all patients, the mean time from disease onset to onset of that clinical feature was calculated and expressed as a percentage of total disease duration.

Pure akinesia with gait freezing
Present:
Gradual onset
Early freezing of gait or speech
Absent:
Sustained response to levodopa
Tremor
Imaging changes suggestive of lacunar infarcts or subcortical white matter ischaemia suggestive of Binswanger's disease
Absent in first 5 years:
Limb rigidity
Dementia
Supranuclear ophthalmoplegia
History of acute focal neurological events due to stroke

Table 6.6 Diagnostic criteria for pure akinesia with gait freezing

Results

Seven cases (five men, two women), fulfilled criteria for pure akinesia with gait freezing. The primary pathological diagnosis was PSP in six and Lewy body pathology in the seventh. One patient with a diagnosis of PSP had previously been classified PNLA because of sparse neurofibrillary tangles on routine silver staining, but subsequent immunohistochemical analysis revealed typical PSP neuronal and glial pathology. Clinical descriptors of gait freezing and gait ignition failure were varied amongst these cases, and could not be delineated from gaits associated with other pathologies. Other clinical features were able to make the distinction between VP and most PD from PAGF. Four patients with isolated vascular disease who presented with slowing of gait and bradykinesia were excluded because of co-existent tremor, rigidity and dementia in four

and history of acute stroke episode in one. In ten patients with combined vascular and Lewy body pathology who presented with slowing of gait and freezing PAGF was excluded by co-existent tremor, rigidity and response to levodopa medications. In one patient with shunt-responsive PSP, diagnosed as normal pressure hydrocephalus in life, PAGF was excluded by early cognitive dysfunction. Patients with isolated Lewy body disease who presented with gait disturbance were excluded by their response to levodopa medications, rigidity and cognitive dysfunction. Table 6.7 lists the red flags for these distinctions.

The clinical features of PAGF are summarised in table 6.8. The mean age at disease onset was 61 years (range 44-78) and mean disease duration was 13 years (range 5-21). The natural history and evolution of clinical features in these patients is summarised in figure 6.3. Akinesia of gait, speech and poverty of facial expression were common early features. Back pain and nuchal rigidity were also reported. Most patients were wheel chair dependent in the second half of their disease, and eye movement abnormalities typically occurred after 9 years of disease.

Of the six cases with a pathological diagnosis of PSP, three had been suspected to have PSP, a mean of 11 years after disease onset, and three had carried a final clinical diagnosis of PD. Five of the cases had been classified as PSP-P in chapter 6.1. In the patient without PSP pathology there was a well documented history of dementia and visual hallucinations in the last 8 years of disease but other clinical features were not substantially different from the PSP cases.

Disease duration was longer in pure akinesia with gait freezing than in other archived cases of PSP (PSP-PAGF 11.9 vs. PSP 8.0 years, *t-test p=0.034*). These differences were significant when PSP-PAGF was compared to RS, though there was no significant difference when compared to PSP-P. (Table 6.8) There were no significant differences between these groups in age of onset or age at death. There were no significant differences in age of onset or death between PSP-PAGF and other Parkinsonian conditions, except that in cases of CBD, disease duration was shorter (PAGF 11.4 vs. CBD 6.8, *t test p=0.01*).

PAGF	Fast micrographia Rapid hypophonia Palilalia
PD	Associated rigidity Rest tremor Response to levodopa Quiet dysprosodic slurred speech Visual hallucinations
VP	Leg rigidity Tremor Early cognitive dysfunction Pyramidal signs History of acute stroke

Table 6.7 Red flags for distinguishing PAGF from PD and VP

	Case number						
	1	2	3	4.	5	6	7
Age at onset (yr)	68	44	63	49	71	78	55
Age at death (yr)	81	60	75	62	76	87	76
Disease duration (yr)	13	16	12	13	5	11	21
Gender	F	F	M	M	M	M	M
First symptom	Gait disturbance	Slowed gait	Slowed gait	Speech disturbance	Slowed gait	Slowed gait	Gait disturbance
Time to first fall (yr)	5	4.5	-	8	?	8	?
Time to wheelchair (yr)	11	-	7	7		7	
Speech	Hypophonia	Hypophonia		Stammering, rapid hypophonia	Rapid hypophonia	Hypophonia	Dysarthria
Handwriting	-	Micrographia	Micrographia	Micrographia	-	-	-
Upper limb bradykinesia	Late	No	No	Late	Mild	No	Late
Time to gaze paresis (yr)	8	13	-	5	-	-	-
Eye signs	Bleph H & V SNGP	Bleph Late SNGP	Normal	Bleph V then H SNGP	Bleph	Normal	Hypometric saccades
Cognition	Normal	Normal	Normal	Normal	Normal	Normal	Late frontal dysexecutive

Table 6.8 Demographic features F, female; M, male; ?, unknown; H, horizontal; V, vertical; SNGP, supranuclear gaze palsy; -, not relevant; Bleph, blepharospasm

	Age of onset (range) *years*	*p*	Age at death (range) *years*	*p*	Duration (range) *years*	*p*
PSP-PAGF	62.2 (44.4-78.1)		73.6 (60.9-87.0)		11.4 (5.5-16.5)	
PSP	65.7 (40.1-87.5)	NS	73.7 (45.4-95.8)	NS	8.0 (1.2-23.8)	0.034
RS	66.0 (40.1-87.5)	NS	72.3 (45.4-95.8)	NS	6.3 (1.2-13.5)	<0.001
PSP-P	65.8 (44.3-86.1)	NS	76.4 (50.1-92.6)	NS	10.5 (4.1-23.8)	NS
LBP	61.0 (28.6-84.2)	NS	76.3 (49.8-93.4)	NS	15.1 (1.4-41)	NS
MSA	56.4 (33.5-79)	NS	64.4 (39.4-86.4)	NS	7.9 (2.9-16.5)	NS
CBD	66.2 (55.6-76.1)	NS	73.0 (65.8-81.6)	NS	6.8 (3.6-10.2)	0.01
VP	71.7 (59.7-81.3)	NS	82.4 (72.4-90.8)	NS	9.9 (2.6-18.2)	NS

Table 6.9 Mean age of onset and disease duration in PSP-PAGF vs. other bradykinetic rigid syndromes. *p*, Student's t-test p value compared to pure akinesia; NS, non-significant.

Case histories:

Case 1 – Pathological diagnosis: PSP

This 68 year old woman presented to her local doctor with low back pain and progressive difficulties walking because of feelings of unsteadiness. Degenerative lumbar disc disease was diagnosed 3 years later, after she experienced an acute exacerbation of lumbar and buttock pain. Her gait disturbance deteriorated and the following year she was found to have marked difficulty initiating walking, but she was able to walk fluently once she had got started. There was mild leg rigidity and no tremor. Cognition and eye movements were normal. There was a mild, unsustained improvement when L-dopa was tried four years after disease onset. However, by the following year there was no response to a daily dose of 1500 mg of l-dopa/carbidopa, and she had a quiet speech, had slowed up mentally and required a walking stick for support. Examination, five years after disease onset, revealed a slowing of vertical saccadic eye movements but no supranuclear gaze palsy. Over the next 2 years, however, there was gradual deterioration in the range of her eye movements, and she developed swallowing difficulties. Her gait became so unsteady that she was unable to walk without two people supporting her and at home she

was confined to a wheel chair. There was neither delirium nor visual hallucinations and her memory was normal. When re-examined at 79 years of age she had blepharospasm and gaze limitation in the horizontal and vertical planes. There were no frontal lobe release, pyramidal or cerebellar signs. When she died 2 years later she had been resident in a nursing home for 4 years and was dependent on others for most activities of daily living.

Case 2 - Pathological diagnosis: PSP

A 44 year old woman was referred to an orthopaedic surgeon following a nine month history of aching thigh pain, particularly at night. Over the next 2 years she developed facial impassivity and slowness of gait with decreased arm swing. Examination revealed marked neck rigidity but normal tone in the limbs and there was no rest tremor. Anticholinergic medications were ineffective. In the following year she developed some dystonic posturing of the left foot on and after walking. L-dopa/carbidopa 220 mg was started without improvement. She became increasingly unsteady, and she developed a specific difficulty in initiating walking, but was able to walk with normal stride length once started. By the time she was 50 she was falling frequently, and experienced gait freezing when walking through doorways. Amantadine 200 mg daily had no effect. The following year she developed a quiet slurred speech, facial freezing and micrographia but no other signs of Parkinsonism in the arms. She continued to fall frequently and 11 years after disease onset she was confined to a wheelchair when venturing out of doors and used a single point stick indoors. Her cognition was unaffected, and apart from handwriting difficulties upper limb function was normal. Selegiline and bromocriptine were ineffective, and the latter precipitated visual and auditory hallucinations which disappeared on withdrawal. She developed pyramidal signs and by 57 years of age had blepharospasm and some restriction of her vertical gaze. Ocular motility deteriorated over the next 2 years until voluntary eye movements became impossible. Terminally she developed laryngeal dystonia, stridor and dysphagia leading to frequent respiratory compromise and depression. She became delirious and died suddenly from presumed respiratory failure at the age of 60.

Case 3 - Pathological diagnosis: PSP

This 63 year old man gradually developed speech disturbance and slowness of walking. One year later his handwriting deteriorated and he was found to have moderate bradykinesia in the limbs, worse on the right than the left. L-dopa/carbidopa was started without clear benefit up to 1000 mg per day. The bradykinesia increased and selegiline and bromocriptine were added but with no improvement. Within five years he had developed start hesitation and difficulties moving off from a standing start. Increasingly over the next two years he experienced gait initiation failure and freezing. He required a single point stick to mobilise indoors and a wheelchair outside. Cognition was unaffected, and, apart from micrographia, he was able to use his hands for everyday tasks without difficulty. Walking became impossible by 72 years of age and he became dependent on a wheelchair to get around. There was no rigidity in the limbs. Further trials with dopaminergic medication were ineffective. He developed depression after being diagnosed with bladder cancer and died from complications of the cancer at 75 years of age.

Case 4 - Pathological diagnosis: PSP

This 49 year old man presented to his doctor with depression and phobic anxiety. He had developed speech difficulties and deterioration in his hand writing. He complained of a stutter and his speech would sometimes suddenly block in mid-sentence. The family also commented that his speech was faster and more imprecise. Three years later he had developed facial impassivity and walked very slowly. L-dopa/benserazide (Madopar) was started four years after onset with no improvement, up to 1200 mg per day. He tended to freeze in crowds, and over the next year developed gait initiation difficulties. There was no tremor or rigidity. His start hesitation and blocking increased and he was referred for consideration of pallidotomy. He had normal cognition and normal function in the arms. The right sided stereotactic pallidotomy was complicated by aphonia and produced no benefit to his gait. His condition deteriorated further over the following four years, and he developed blepharospasm and a complete supranuclear gaze palsy, initially in the vertical plane. In the final stages of his illness he had severe limb rigidity and died after 13 years of disease from presumed aspiration pneumonia.

Case 5 - Pathological diagnosis: PSP

This 71 year old presented with a gradual onset of difficulty initiating movements and speech disturbance. His speech had become quiet and his handwriting much smaller. L-dopa/carbidopa was started and increased to 660 mg per day without noticeable effect. Three years later he had a rapid, monotonous, low volume speech and profound gait initiation problems with shortened stride length when he started walking, but good arm swing. There was a mild decrement in amplitude on sequential finger movements, but no limb rigidity, tremor or hypomimia. He then started to complain of freezing up in doorways and confined spaces. He developed an anterocollis and mild swallowing difficulties. He had no rigidity and only minimal bradykinesia in the arms right up to the final year of his life. His speech deteriorated to the point where he was virtually aphonic, only able to communicate by speaking slowly in a whisper. Though no formal neurological examination was performed in the last months of his life he is recorded to have had a staring expression with a tendency for his eyes to spontaneously close.

Case 6 - Pathological diagnosis: PSP

At 78 years of age this man gradually developed unsteadiness and slowness of gait. His walking progressively deteriorated, over the next three years and he was referred to a neurologist who recorded gait initiation problems, shuffling gait and freezing. There was a moderate hypophonia, but no bradykinesia, tremor or rigidity in the arms. There was no response to l-dopa/carbidopa or bromocriptine. After five years disease duration he developed dysphagia and his speech became unintelligible. No cognitive dysfunction or oculomotor abnormality was described at the last clinical visit 2 months before his death at 87 years of age.

Case 7 - Pathological diagnosis: PD

At 50 years of age this man noticed progressive unsteadiness, poor balance and eventually difficulty initiating walking and turning. He had history of traumatic brain injury and was comatose for two weeks following. There was also a history of long and liberal alcohol intake. He started L-dopa/benserazide 625 mg without benefit and the

medication was withdrawn, and there was no response to 3 mg of subcutaneous apomorphine. An MRI scan did not show any evidence of vascular disease or hydrocephalus. At lumbar puncture the cerebrospinal fluid (CSF) pressure was normal, and there was no improvement following three large volume CSF taps. After 10 years he developed some urinary hesitancy and terminal dribbling. Even after 20 years there were no signs of Parkinsonism in the upper limbs, only mild hypomimia and normal eye movements. He scored 30 on Folstein's mini mental state examination, but on detailed neuropsychometric testing there was evidence of moderate under functioning of non-verbal reasoning tasks. Subsequent treatment with selegiline and amantadine was ineffective. In the final years of his illness there was dystonic posturing of the right arm, apraxia and fluctuating cognitive state with hallucinations and daytime somnolence.

Discussion

Pure akinesia is an uncommon presentation of PSP-tau pathology and is clinically distinct from the more common PSP phenotypes of RS and PSP-P. The striking consistent clinical features are difficulties with the initiation of walking and freezing of gait with associated handwriting and speech difficulties. In contrast to PSP-P limb rigidity and tremor are absent and cognition remains intact. In common with PSP-P eye movement abnormalities emerge late in the disease. We have identified seven cases in a brain bank cohort of 753 patients with a presentation of start hesitation and freezing of gait. These cases resemble the patients first described by Imai (Imai and Narabayashi, 1974) and subsequently documented in several small case series by other authors. In a number of later reports similar cases have been referred to as gait ignition failure or primary progressive gait freezing. (Yamamoto *et al.*, 1985; Imai *et al.*, 1993; Atchison *et al.*, 1993; Riley *et al.*, 1994; Factor *et al.*, 2002) We would like to propose "pure akinesia with gait freezing" as a unifying term which encompasses the salient clinical features.

Six of the patients had the pathological features of PSP, one of whom had previously been labelled as pallidonigro-luysian atrophy (PNLA), and a seventh had Lewy body pathology. A natural history of pure akinesia with gait freezing can be constructed from the retrospective analysis of patients in our study (figure 6.3). Start hesitation and a change in handwriting or, less frequently speech, are common presenting

features. Other early complaints include unsteadiness and slowing of gait, untidy handwriting and mildly slurred speech. As the disorder evolves, gait ignition failure and freezing, rapid stuttering and hypophonia with fast 'pallidal' micrographia occur. Some patients develop axial rigidity and back pain, and later bladder instability. Supranuclear gaze paresis and blepharospasm are late and inconsistent features. Death usually occurs more than a decade after disease onset by which time some patients have developed additional upper limb bradykinesia and swallowing problems. (Petren, 1901; Achiron *et al.*, 1993; Atchison *et al.*, 1993; Factor *et al.*, 2002; Factor *et al.*, 2006)

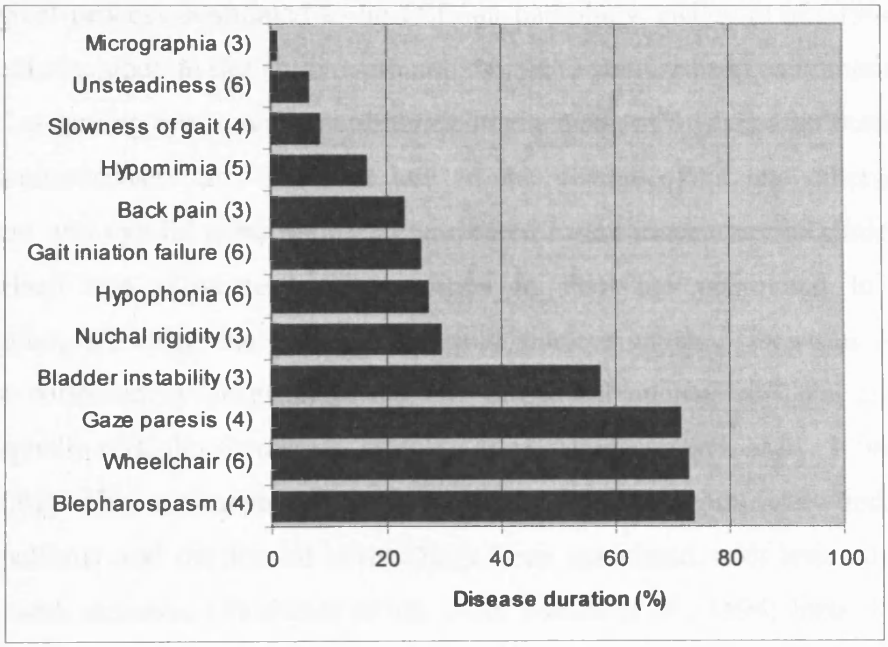


Figure 6.3 Natural history of PSP-PAGF, summarised clinical features from 6 patients showing mean time from disease onset (expressed as percentage of total disease duration, *x*) to onset of clinical feature (*y*)

Amongst the patients with a pathological diagnosis of PSP in this series, pure akinesia with gait freezing (PSP-PAGF) can be differentiated from RS by the absence of a frontal dysexecutive syndrome, eye movement abnormalities and falls within the first two years of disease. In chapter 4.1 patients with PSP-PAGF were grouped together with PSP-P. PSP-PAGF can, however, be further separated from PSP-P by the presence of

gait ignition failure, rapid hypophonia or micrographia and the absence of levodopa response and tremor. The duration of disease is similar to PSP-P but is longer than in RS.

An association between PAGF and PSP-tau pathology was first suggested after the recognition of late eye movement abnormalities. (Imai *et al.*, 1987) Abnormalities of vertical downward saccadic eye movements are considered to be most specific for the diagnosis of PSP and, in RS eye movement abnormalities often occur within the first two years of disease. (Litvan *et al.*, 1996a) In a clinically diagnosed group of patients with pure akinesia, eye movement recordings showed slowness of vertical saccades that was proportional to disease duration and hence likely to be a product of the underlying pathological process postulated to be PSP-tau pathology. (Riley *et al.*, 1994) Although the patients included in our study were not subject to standardised examination, five were recorded as having eye movement abnormalities a mean of 8 years after disease onset. In four, blepharospasm also occurred late in the disease. This late emergence of eye movement and eye-lid abnormalities contributed to the inaccuracy of clinical diagnosis. The earliest eye movement abnormalities in PSP are postulated to result from degeneration affecting the rostral interstitial nucleus of the fasciculus longitudinalis medialis, followed by progression into the interstitial nucleus of Cajal and the central mesencephalic reticular formation. (Revesz *et al.*, 1996; Rottach *et al.*, 1996; Bhidayasiri *et al.*, 2001) The neuroanatomical basis for akinesia is less completely understood. The globus pallidus and the frontal cortex have been associated with lesional and imaging studies with akinesia. (Taniwaki *et al.*, 1992; Kondo *et al.*, 1994; Imai, 1996; Ohno *et al.*, 1997; Kim *et al.*, 2001; Yener *et al.*, 2005) Pathological studies have consistently shown involvement of the globus pallidus and substantia nigra and in most cases the subthalamic nucleus, (Homma *et al.*, 1987; Matsuo *et al.*, 1991; Mizusawa, 1993; Yoritaka *et al.*, 1997; Katayama *et al.*, 1998; Konishi *et al.*, 2005) and a number of cases of secondary pure akinesia have been attributed to structural lesions in the globus pallidus. (Suzuki *et al.*, 1984; Pramstaller *et al.*, 1999)

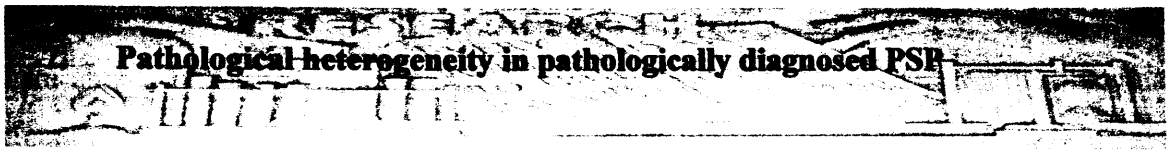
Lower-body Parkinsonism has been linked to a vascular aetiology for more than a century (Brissaud, 1895), and accounts for around 4% of all Parkinsonism. (Foltynie *et al.*, 2002) However, the clinical diagnosis of VP is often inaccurate (Hughes *et al.*, 1992b; Jellinger, 2002), and clinical correlates of VP are limited to few large autopsy

series. (Hughes *et al.*, 1992b; Jellinger, 2001; Zijlmans *et al.*, 2004a) In the current series no patient with pathologically diagnosed VP, or PD with added vascular pathology, satisfied the criteria for PAGF. A number of patients with VP presented with early gait freezing, marche à petits pas, micrographia and hypophonia, but the presence of tremor, dementia response, limb rigidity and a sustained response to dopaminergic medications, excluded them from PAGF according to our criteria. Furthermore these additional clinical features are typical for VP, suggesting that the clinical diagnostic criteria would exclude most VP from a diagnosis of PAGF. (Winikates and Jankovic, 1999; Zijlmans *et al.*, 2004b) Lewy body pathology only accounted for one patient in our series, and is differentiated from the other cases only by the late onset of dementia and visual hallucinations. Postural instability and gait disturbance are poor prognostic features in PD, and suggest an early progression to dementia. (Burn *et al.*, 2006; Alves *et al.*, 2006) In general these patients are unlikely to be classified as PAGF because of responsiveness to dopaminergic medications and rigidity which tends to be more severe than in PD patients with tremor dominance. (Giladi *et al.*, 2001)

The goal in defining any disease is "to gain insight into its nature, with the implicit assumption that disease exists as an ideal form". (Nesse, 2001) The proposed diagnostic criteria for pure akinesia with gait freezing emphasises the absence of limb rigidity and rest tremor and the absence of any substantial, sustained response to levodopa. Some of the published cases of pure akinesia, primary progressive gait freezing, gait initiation failure and Petrén's gait would be reclassified using these criteria, presumably as vascular Parkinsonism or PD. A number of rare causes have been reported to cause secondary pure akinesia, involving brain regions known to be affected in PSP. Sarcoma in the caudate, globus pallidus and thalamus (Suzuki *et al.*, 1984), primary central nervous system lymphoma involving the globus pallidus bilaterally (Pramstaller *et al.*, 1999), pheochromocytoma (Nakagawa *et al.*, 1987) and genetically proven Hallevorden-Spatz syndrome (Molinuevo *et al.*, 2003) have caused similar symptoms, but the tempo of disease and imaging characteristics would have excluded PSP-tau pathology in these cases.

The findings suggest that PSP pathology is the most common underlying pathological process in patients with pure akinesia with gait freezing. A clinically

identical syndrome can also be associated with Lewy body disease, although the presence of rigidity and dementia with visual hallucinations might separate these cases in the later stages of the disease. The proposed diagnostic criteria for pure akinesia and gait freezing will improve the clinical and pathological homogeneity, and hence clinical utility of this clinical syndrome.



Introduction

The diagnosis of PSP can only be made with absolute conviction after post mortem confirmation. (Litvan *et al.*, 1996a; Osaki *et al.*, 2004) Clinical variability in disease accounts for the low specificity and sensitivity of diagnostic criteria. (Osaki *et al.*, 2004) The different clinical expressions of PSP-tau pathology suggest that there might be regional variability of pathological change. Several pathologists have addressed this issue by examining the distribution and severity of pathological changes in series of cases where the minimum pathological criteria for PSP are satisfied. Unfortunately the accompanying clinical descriptions in these series are often incomplete and the definition from report to report of "atypical" PSP varies.

Regional pathology in PSP

In one series Braak et al., demonstrated that patients with early, severe dementia have relatively more cortical pathology. (Braak *et al.*, 1992) They found a correlation between dementia and severity of cortical lesions and emphasised the need to consider PSP as disease with significant regional heterogeneity. In the same year Hof et al., quantitatively analysed the brains of 6 patients with PSP to determine the distribution of cortical neurofibrillary tangles. (Hof *et al.*, 1992) A variable distribution of pathology and different densities of neurofibrillary tangles in the prefrontal, primary motor and temporal cortices were found. However, pathological changes in the granule cell layer of the dentate gyrus were uniform.

Later Vermersch et al., eloquently mapped the pattern of neurofibrillary degeneration in one patient using biochemical and immunohistochemical methods. (Vermersch *et al.*, 1994) The greatest tau load was detected in the motor strip and prefrontal areas. Verny et al., examined in detail the distribution of tangle formation and neuronal loss in 10 cases of PSP. (Verny *et al.*, 1996a) Using morphometric analysis, the mean density of NFTs was calculated using contiguous microscopic fields, transferred to

fit a 10-level standard scale. They performed a factorial analysis, using these results, and were able to identify two subgroups that could be divided on clinical and pathological grounds. A group with mild involvement of the pedunculopontine nucleus invariably had associated mild cortical involvement and a number of clinical signs that they considered unusual, including rest tremor at presentation, blepharospasm and facial dystonia, myoclonus on startle and postural tremor in different patients. The subcortical structures involved had a preferentially 'pallido-luyso-nigral' distribution. The second group all had typical clinical signs and more severe involvement of the pedunculopontine nucleus and cortex, and more widespread involvement of subcortical structures. The sample size was too small to be conclusive about a true difference between these groups.

The presence or absence of a described vertical supranuclear gaze palsy was used to define clinical groups by Revesz and colleagues at the National Hospital for Neurology, Queen Square. (Revesz *et al.*, 1996) They identified changes in the degree of nerve cell loss and neurofibrillary tangle deposition in the nucleus raphe interpositus (nRI) which contains the omnipause neurons. The classic clinical group with supranuclear gaze palsy had the most severe changes in the nRI, which has a role in the initiation of saccadic eye movements.

The neuropathological characteristics of 3 patients with "clinically atypical" PSP were reported by Bergeron et al. (Bergeron *et al.*, 1997) These patients had developed a corticobasal syndrome, with apraxia, focal dystonia and arm levitation late in the disease. Neuronal loss and gliosis, tau immunoreactive threads, neuronal tau inclusions and tau immunoreactive astrocytes in the cerebral cortex, subcortical structures and cerebellum were found and pathological changes were most severe in the frontal lobes. Bigio et al., found similar changes in two groups, where the atypical PSP group was defined as having severe dementia at death. (Bigio *et al.*, 1999) Using a manual semi quantitative 4-grade scale they found a positive association between severity of cortical tau pathology and dementia.

Takanashi et al., compared the globus pallidus and frontal lobes of six cases of PSP and five of CBD, using a 4-grade scale to measure the expression of pathological tau. (Takanashi *et al.*, 2002) They found very few changes in the frontal cortices in five of the six (83%) PSP cases, but the clinical details of were not reported.

Variability has been reported in the substantia nigra (SN) pars reticulata lesions in six cases of PSP. (Halliday *et al.*, 2000) The topography and degree of cell loss within was analysed using serial section analyses and unbiased quantitative techniques. Significantly more reticulata neuronal loss was reported in the 4 cases with gaze palsy.

Halliday and her colleagues have also examined the globus pallidus (GP), subthalamic nucleus (STN) and SN in PSP and PD. (Hardman *et al.*, 1997a; Hardman *et al.*, 1997b; Hardman and Halliday, 1999a; Hardman and Halliday, 1999b) Neuronal numbers were calculated using an unbiased fractionator technique following immunostaining. The density of NFTs and neuropil threads was quantified to obtain severity index on a 4-grade scale and neurones were counted in each nucleus. Significant, but variable neuronal loss was found in the GP externa of the PSP cases, but not PD. All other regions examined in the PSP cases had substantial (>60%) neuronal loss.

Regional variability has also been noted in the cerebellar cortex of 13 cases of PSP. (Piao *et al.*, 2002) Tau positive pre-tangles were found in the cerebellar cortex in the absence of NFTs in 9/13 (69%), but no clinical correlations were suggested.

Substantial regional variability in NFT and pre-tangle distribution was reported in another study. (Li *et al.*, 1998) Tau positive neurons (NFT & pre-tangles) were counted in five cortical (superior frontal, precentral, entorhinal and transentorhinal cortices and the hippocampus) and five subcortical (amygdala, Meynert nucleus, hypothalamus, pallidum, subthalamus) regions in eight cases. In these regions up to three fold differences in the density NFT were reported. The group of patients with the highest number of pre-tangles, was designated 'atypical PSP' on the basis that pre-tangles are rare in PSP, but common in CBD. In their 'typical' group, with few pre-tangle neurons, NFTs were most abundant in the STN, GP, Meynert nucleus, central nucleus and hypothalamus.

In a post mortem imaging study using nine pathologically confirmed cases of PSP, regional brain atrophy (Cordato *et al.*, 2000) was greatest in the GP interna, the amygdala, frontal lobes and parietal lobes compared with controls and PD. Interestingly the degree of atrophy of the GPi and amygdala correlated with increasing tangle formation in the precentral gyrus.

Regional analysis of tau isoform accumulation in PSP

Delacourte and colleagues demonstrated selective vulnerability of neuronal subsets in PiD and AD that corresponded to the expression of specific tau isoform patterns. (Delacourte *et al.*, 1996; Delacourte *et al.*, 1998) They suggested that tau accumulation could perform as a biochemical marker for "neuronal subsets and their specifically related degenerative processes". (Delacourte *et al.*, 1996) Later in their seminal paper on the biochemical pathway of AD, they used these methods to demonstrate a hierarchical 'collapse' of subsets of neurons using paired helical filament (PHF)-tau as a marker. (Delacourte *et al.*, 1999)

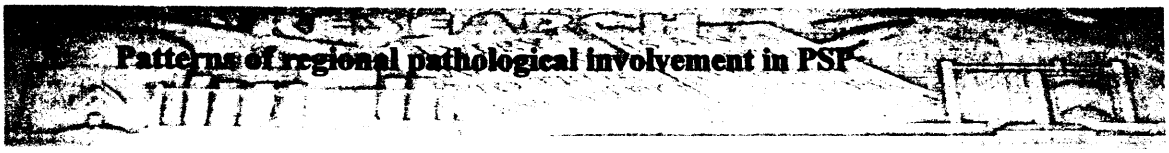
In PSP tau deposition was first mapped biochemically in 1994. Vermersch sampled several subcortical regions, brain stem regions, and all cortical areas using Brodmann classification. (Vermersch *et al.*, 1994) These were analyzed using an anti-PHF-tau antibody and western blotting, and band intensity was measured by computerised densitometry. Abnormal doublet tau was found in all cortical and subcortical regions. The largest amount of tau was detected in the motor strip and prefrontal areas.

Regional analysis of tau expression was later performed using tau real time – polymerase chain reaction (PCR) in 15 cases of PSP using tissue taken from the frontal cortex, cerebellar cortex and brainstem (including midbrain and medulla). (Chambers *et al.*, 1999) An increase in 4R tau mRNA in the brainstem was found in these cases, but not in the frontal cortex or cerebellum. Weak expression of 3R mRNA in the cerebellar cortex was also noted.

Takanashi *et al.*, performed the first comparative study of quantitative analysis of tau mRNA isoforms in pathologically diagnosed cases of PSP, CBD and controls. (Takanashi *et al.*, 2002) There was no difference in total amounts of 4R and 3R tau between the three groups, but a high ratio of 4R/3R tau mRNA was reported in the globus pallidus of PSP and CBD cases, and in the frontal lobes of CBD cases only.

Conclusion

Pathological heterogeneity has been reported in PSP within the accepted pathological diagnostic criteria. The differences are found in: the degree of neuronal loss in the frontal lobe, temporal lobe, pontine base, periaqueductal grey matter, nRI, SN pars reticulata, GP, dentate nucleus and cerebellar cortex; and there is also variability in the distribution of NFTs and pre-tangles in the superior frontal-precentral cortex, amygdaloid central nucleus, GP, STN and dentate nucleus. The clinical importance of these changes is unclear. This heterogeneity does, however, provide the framework on which a structured neuropathological examination can be performed in a group of patients who have been carefully clinically examined in life.



Pathological tau lesions were analysed in 42 cases of pathologically diagnosed PSP (23 RS, 13 PSP-P and 6 PAGF) using specific antibodies for hyperphosphorylated tau (AT8, RD4 and RD3). The severity of tau-positive lesions (neurofibrillary tangles, tufted astrocytes, coiled bodies and neuropil threads) was graded in 20 brain regions. The mean overall severity of AT8 positive lesions was higher in RS than in PSP-P ($p<0.001$) and in PAGF ($p<0.001$). In all three clinical syndromes tau pathology was most severe in the subthalamic nucleus (STN), substantia nigra (SN) and globus pallidus interna (GPi). In RS, tau-pathology was significantly more severe than PSP-P in all regions ($p<0.05$) except the STN and putamen, and more severe than PAGF in the parietal cortex, pons, dentate nucleus and cerebellar white matter ($p<0.05$). Three patterns of distribution of pathological tau were identified by cluster analysis: in cluster 1 the burden of tau accumulation was highest in the STN, SN and GPi, and relatively spared the other regions, accounting for most cases of PSP-P; in cluster 2, in addition to the basal ganglia, there was moderately severe tau pathology in the pons, cerebellar white matter and some cortical regions; and in cluster 3 there was moderately severe tau pathology in most cortical regions and basal ganglia and more severe pathology in the pons, dentate nucleus and cerebellar white matter. Clusters 2 and 3 accounted for the majority of RS patients. The incidence of Alzheimer's type pathology, Lewy bodies and vascular pathology did not differ between clinical groups. The findings of this study suggest that the 'atypical' clinical features of PSP-P and PAGF are underpinned by PSP-tau pathology that is less severe and less widespread than in RS.

"...if I were to describe in a single word the services rendered to us by... pathological anatomy...I should say that it has taught the physician to think anatomically"

(J. M. Charcot. Maladies des vieillards. In: Oeuvres complètes de JM Charcot. Tome VII. Paris: Lecrosnier et Babé, 1890. [Translated into English by W.S. Tuke. Clinical lectures on senile and chronic disease by J.M. Charcot. London: The New Sydenham Society)

Aims

To examine the distribution of PSP tau pathology, specifically with a view to determine what extent pathological variability might explain the clinical differences between RS, PSP-P and PAGF.

Materials and Methods

We selected 42 cases, from 102 pathologically diagnosed as PSP, that were archived at the QSBB between 1992 and 2002. A systematic case note review and clinical classification (RS, PSP-P and PAGF) was performed as described in chapter 5. The analysis started with the most recently archived 21 cases comprising 13 cases of RS, seven of PSP-P and one of PAGF. A further 21 were selected according to clinical phenotype. This second group comprised nine cases of RS and seven cases of PSP-P randomly selected from the remaining 81 cases and five cases of PAGF.

Pathological Methods

Diagnostic procedures

Consent for brain donation was obtained from the patient prior to death and consent for post mortem examination was obtained from the next of kin after death. After removal the brain was divided in the midline and one half was frozen and stored at -80°C. The other half was fixed in 10% formalin and sliced in the coronal plane. The routine analysis of post mortem material at QSBB included removal of tissue blocks from the frontal, temporal, parietal and occipital neocortices, in addition to basal ganglia, thalamus, amygdala, hippocampus, midbrain, pons, medulla and cerebellum. All brain areas were processed in paraffin wax using standard protocols. Routine sections were cut at 7μm and stained with routine methods including haematoxylin and eosin (H&E), luxol fast blue/cresyl violet (LFB/CV), tau or silver impregnation (modified Bielschowsky's method). Preliminary PSP pathological diagnostic criteria were applied (Litvan *et al.*, 1996b) which insist on the presence of neurofibrillary tangles (NFT), gliosis and nerve cell loss in the subthalamic nucleus (STN), pallidum, substantia nigra (SN), dentate nucleus, inferior olive and oculomotor nuclear complex.

Immunohistochemistry

In each case selected for this study, 7 μm tissue sections were taken from the anterior and posterior frontal cortex, parietal and temporal cortices, midbrain, griseum pontis, and cerebellum for immunohistochemical analysis with the AT8 (tau phospho-epitope Ser202/Thr205; Autogen Bioclear, Calne, UK), and three-repeat specific (RD3, Upstate, Dundee, UK) and four-repeat-specific (RD4, Upstate, Dundee, UK) anti-tau antibodies. Tissue sections were coded with a novel identifier to allow for examination blinded to clinical details. The sections which were stained with RD3 and RD4 required pre-treatment pressure cooking in sodium citrate buffer (pH 6) for 10 minutes. They were then incubated with RD3 (1:1000) or RD4 (1:100) at room temperature for 1 hour, followed by biotinylated antimouse IgG (1:200, Dako, Cambridgeshire, UK) for 30 minutes. They were then further incubated in ABC (Avidin-Biotin complex, Dako) for 30 minutes at room temperature. Colour was developed with glucose oxidase nickel diaminobenzidine and the sections counterstained with Mayers haematoxylin for 1 minute. For AT8 immunohistochemistry, tissue sections were pre-treated in a microwave oven in sodium citrate buffer for 20 min. Sections were incubated with AT8 (1:600) at room temperature for 1 hour followed by biotinylated anti mouse antibody (1:200, Dako) for 30 min. The remaining steps were as described above.

In addition, tissue sections were taken from the midbrain for α-synuclein immunohistochemistry and temporal, parietal and frontal cortical areas for Aβ-immunohistochemistry. For α-synuclein and Aβ-immunohistochemistry, sections were pre-treated with formic acid for 15 minutes and then pressure cooked as described above. Sections were incubated with α-synuclein (Vector laboratories) 1:50 or Aβ (1:100, Dako) for 1 hour at room temperature followed by biotinylated anti mouse antibody (1:200) for 30 minutes.

Regional pathological examination and quantification

In each case quantitative tau pathology assessment was carried out by one rater blinded to clinical details (DRW), in 20 brain regions: grey and white matter in anterior and posterior frontal lobes, parietal lobe and temporal lobe; globus pallidus internus and externus; dorsolateral and ventromedial putamen (Ozawa *et al.*, 2004); caudate nucleus;

medial, ventromedial and dorsolateral SN (Ozawa *et al.*, 2004); STN; pontine base; dentate nucleus; and cerebellar white matter. In each region AT8, RD4 and RD3 positive neurofibrillary tangles, tufted astrocytes, and oligodendroglial coiled bodies were counted in seven randomly placed high powered microscopic fields (x20 magnification), and neuropil threads were counted within a 10x10 graticule (x20 magnification).

The distribution of absolute numbers of tau lesions in the first 21 cases made comparison between cases and the identification of patterns of tau pathology difficult because of the weighting of cases with very high scores. To make further comparisons possible a five point grading scale was developed for each region. The distribution of absolute counts for tau pathology, in each region, were examined and plotted along the x-axis and ranges for grades 0 to 4 were assigned on the y-axis. Grade 0 was reserved for when tau-positive pathology was absent. If the distribution of counts fitted a logarithmic curve the upper limit of grade 4 was set as the highest number counted, grade 1 the lowest count above zero and other grades on a log scale along the y-axis. If the distribution of counts suggested that a linear model was more appropriate the upper limit of grade 4 was set at the highest value counted and grades 1-3 were set at 25%, 50% and 75% of this highest value. In regions where the range included less than four values, absolute counts were used.

To assess repeatability, counting of the four pathological inclusions (NFT, TA, CB and threads) was repeated in all regions of 10% of cases, selected to represent a range of severities, by the same rater. The repeatability of measures was calculated for each pathological lesion in each region by calculating the intra-rater SD as follows: (standard deviation $(R_{av1}-R_{av2}))/\sqrt{2}$. The mean SD was calculated for each lesion in each region for all four repeated cases, as well as the mean SD of all lesions in each region and the mean total severity of each case.

Histopathological examination

Tissue was examined independently by two neuropathologists (TR and JH) in a standardised way using Bielschowsky's silver impregnation and Consortium to Establish A Registry for Alzheimer's Disease (CERAD) neuropathological criteria for Alzheimer's disease were applied. (Mirra *et al.*, 1991) Immunohistochemistry was used to assess

vascular (cerebral amyloid angiopathy) and parenchymal Aβ-peptide deposition. (Revesz *et al.*, 2003) Tau immunohistochemistry was used in the hippocampal formation to identify the presence of tau-positive grains. (Togo *et al.*, 2002) The frontal lobe, lentiform nucleus and pons were examined for vascular pathology (small vessel atherosclerosis, lipohyalinosis, microaneurysm and arteriolosclerosis) and its sequelae (lacunes; perivascular rarefaction; diffuse white matter attenuation (Binswanger's type). Vascular pathology was graded as mild (occasional vessels affected), moderate (a significant proportion of the small vessels affected; little or no sequelae noted) or severe (a significant proportion of the small vessels affected; obvious sequelae). The presence of associated Lewy body pathology in the substantia nigra was assessed using α-synuclein immunohistochemistry.

Statistical Methods

Spearman's correlation calculation was used to determine the relationship between disease duration, age of onset and age at death and the mean grade of severity of each lesion. The relationship between disease duration and regional severity of each pathological lesion was also investigated.

Cases were classified according to clinical type and overall pathological severity was compared between these groups by calculating the mean grade of tau pathology in all regions for each clinical group. Univariable analyses using χ^2 for categorical and two-tailed *t* test or the Mann Whitney U test, as appropriate, for continuous variables were applied.

A complete data set of pathological variables was available from 34 cases (21 RS, 12 PSP-P and 1 PAGF). To identify patterns of distribution of tau pathology in these cases, a between groups, hierarchical cluster analyses, using squared Euclidean distance measures was performed. 'Relative' mean pathological grades for each region were calculated and used as variables. These values allow for patterns of regional tau distribution to be identified by correcting for overall tau severity, and were calculated by combining the mean grades for NFT, tufted astrocyte, coiled body and thread pathology in that region and dividing by the mean grade for all regions in that case. Data from the temporal lobe grey matter was excluded to remove any influence of co-existent

Alzheimer pathology on the analysis. In order to minimise the number of variables in the analysis the relative mean grades for the ventromedial and dorsolateral putamen were combined and the sub-regions of the SN were combined. Groups of cases with a similar distribution of tau pathology were identified by examination of the dendrogram solution which graphically shows the similarities of cases from the resolution of the cluster combine. The demographics, clinical features, additional pathologies and severity of tau pathology between the identified clusters were compared. Statistical analysis was performed using SPSS for Windows (version 12.0.1).

Results

Demographics

Forty-two patients (62% men) were included in the study. The mean age of onset was 66.5 years (range 44.4-87.5), the mean age at death was 75.6 years (60.9-95.8) and the mean disease duration was 9.1 years (1.2-17.3). Mean disease duration was longer in patients classified as PSP-P and PAGF, and diagnostic accuracy was lower. (Table 8.1) There was a significant negative correlation between age of disease onset and disease duration (Pearson correlation -0.557, $p<0.001$). (Figure 8.1)

	All	RS	PSP-P	PAGF
n	42	23	13	6
Male: Female	26:16	14:9	9:4	4:2
Age at onset *years*	66.5	67.5	66.7	62.2
(range)	(44.4-87.5)	(56.4-87.5)	(51.2-82.1)	(44.4-70)
Age at death *years*	75.6	74.7	76.8	73.6
(range)	(60.9-95.8)	(65.8-95.8)	(66.5-87.9)	(60.9-81.4)
Disease duration *years*	9.1	7.1	10.2*	11.4*
(range)	(1.2-17.3)	(1.2-15.6)	(3.7-17.3)	(5.5-16.5)
PSP as final clinical diagnosis %	67	91	43*	50*
Time from symptom onset to final	5.1	3.8	5.2	10.7*
diagnosis of PSP *years* (range)	(0.6-14.5)	(0.6-7.5)	(0.5-8.7)	(5.5-14.5)

Table 8.1 Demographic details of patients included, * (Student's t-test, *p<0.05 vs. RS*)

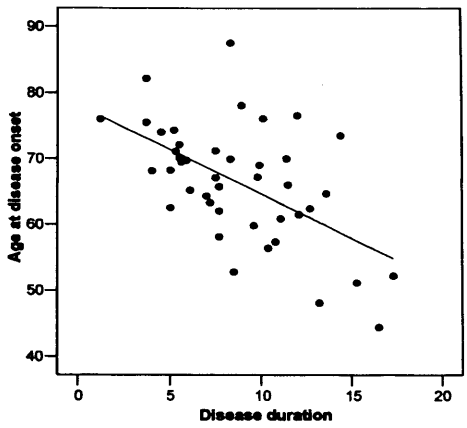


Figure 8.1 Disease duration vs. age at disease onset

Additional pathology

Additional pathological findings are listed in table 8.2. There were no significant differences in the occurrence of AD pathology, Lewy body pathology or vascular pathology between groups. Tau-positive grains were more common in PSP-P (46%) than in RS (18%, Fisher's exact, p=0.084), but not in PAGF (0, Fisher's exact, p=0.126).

	RS	PSP-P	PAGF
Number	23	13	6
CERAD			
Negative	10 (45)	6 (46)	2 (33)
Positive	13 (55)	7 (54)	4 (67)
Sparse	5 (21)	3 (23)	2 (33)
Moderate	7 (30)	3 (23)	2 (33)
Frequent	1 (4)	1 (8)	0
Tau-positive grains	4 (18)	6 (46)	0
Lewy body pathology	1 (4)	0	0
Ischaemic vascular pathology	7 (30)	6 (46)	0
Mild	6 (26)	5 (38)	0
Moderate	0	1 (8)	0
Severe	1 (4)	0	0
Amyloid angiopathy	3 (13)	3 (23)	1 (17)

Table 8.2 Additional pathological diagnoses (%)

Distribution of PSP-tau pathology

The distributions of absolute counts of AT8 positive tau pathology and graded severity are illustrated in figure 8.2. There was no significant difference in the distribution of AT8 and RD4 positive lesions in the first 21 cases. The mean ratio of

AT8:RD4 was 3.25. RD3 positive lesions were not identified in any cases. All further analysis was performed using only AT8 positive lesions. AT8 positive NFT were most abundant in the STN, SN, pontine base, GPi and dentate nucleus. (Figure 8.2A) AT8 positive TA were most abundant in the cortical grey matter and striatum. (Figure 8.2C) The range of values for CB and threads was greater than NFT and TA, and they were most abundant in the STN, SN and GPi. (Figures 8.2E and 8.2G) The distribution of these findings remained after the grading scale was applied, confirming this scale as reasonable. (Figures 8.2B, D, F, H and Figure 8.3)

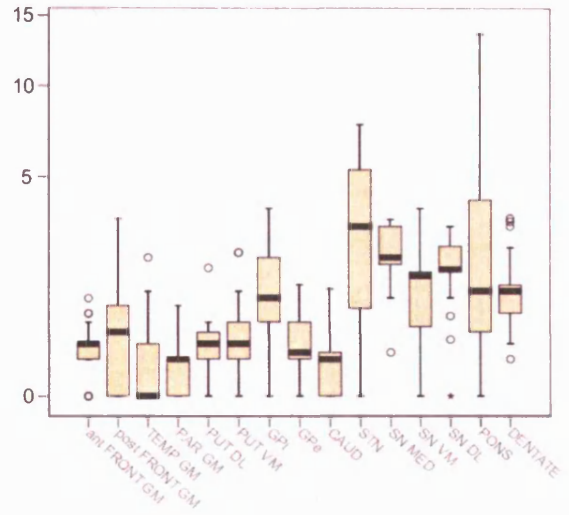


Figure 8.2A Regional NFT counts (21 cases)

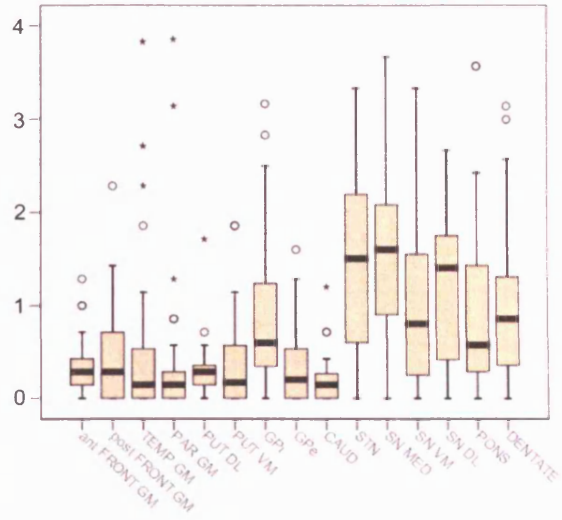


Figure 8.2B Regional NFT grades (all cases)

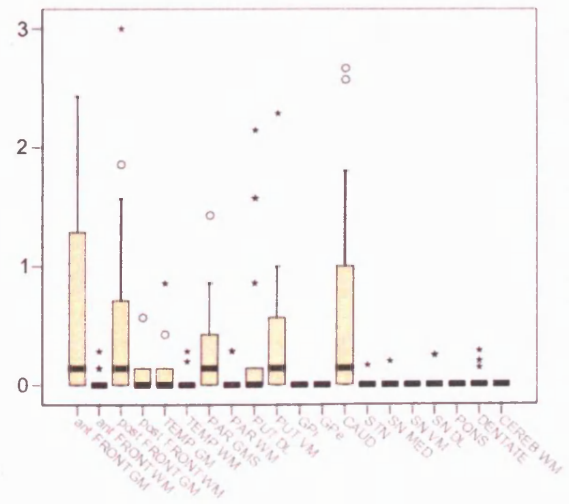


Figure 8.2C Regional TA counts (21 cases)

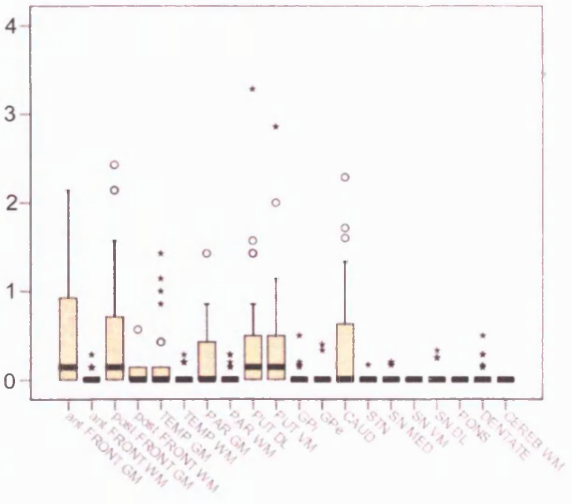


Figure 8.2D Regional TA grades (all cases)

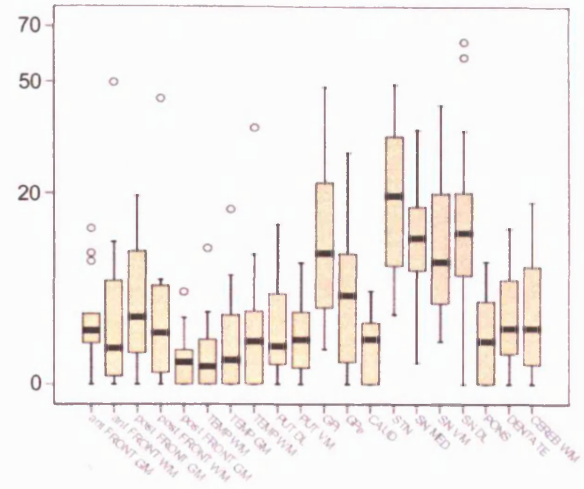


Figure 8.2E Regional CB counts (21 cases)

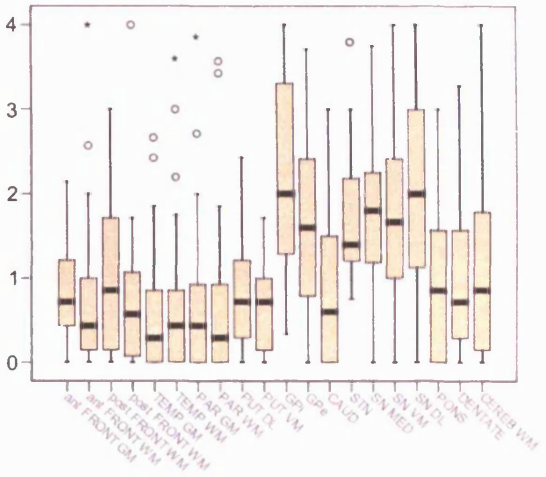


Figure 8.2F Regional CB grades (all cases)

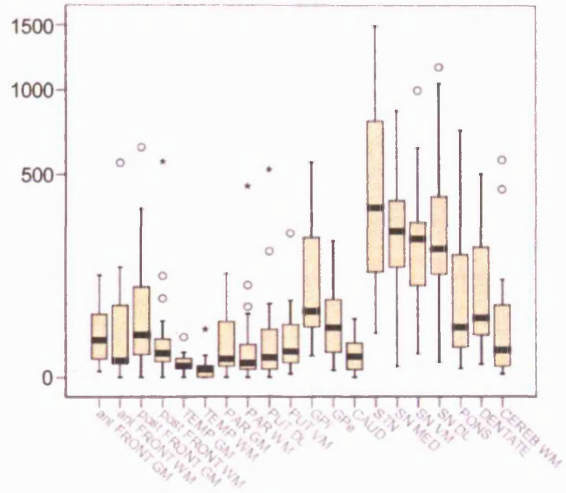


Figure 8.2G Regional thread counts (21 cases)

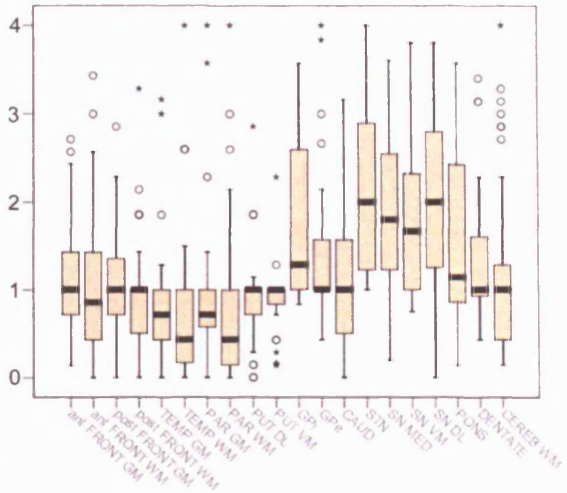


Figure 8.2H Regional thread grades (all cases)

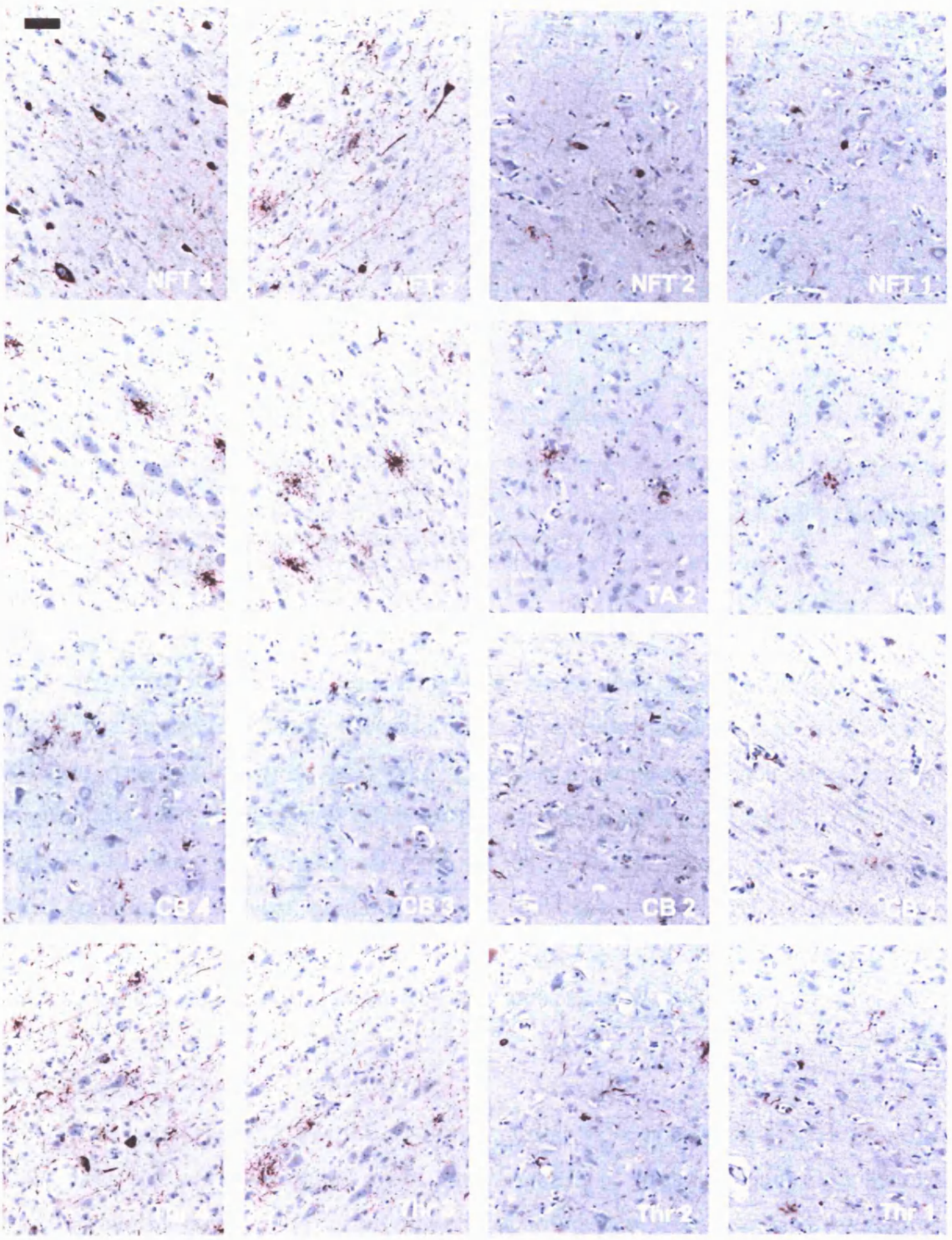


Figure 8.3 Tau lesion severity in the posterior frontal cortex: NFT, neurofibrillary tangle; TA tufted astrocyte; CB, coiled body; Thr, thread pathology; Grades 1 (least severe) to 4 (most severe)
Bar represents 50 μm on all panels. (x20 magnification)

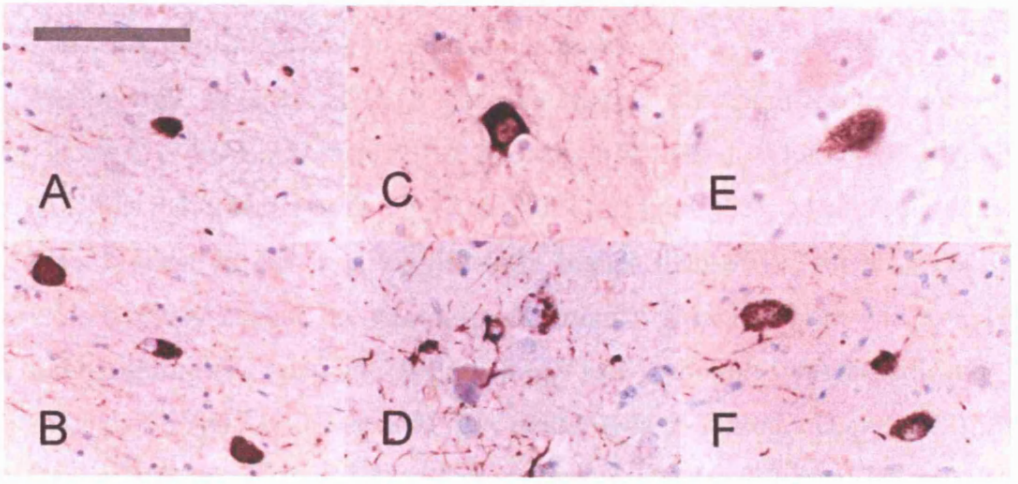


Figure 8.4 Microphotographic illustrations showing the difference in tau-load between cases of PSP-PAGF (A, C, E) and RS (B, D, F).
(A & B) subthalamic nucleus, (C & D) posterior frontal cortex, (E & F) cerebellar dentate nucleus tau immunohistochemistry (AT8 antibody).
Bar on A represents 30 μm on all panels. (x20 magnification)

No correlation was identified between overall mean grade of severity for each pathological lesion (NFT, TA, CB and threads) and disease duration, age at onset or age at death. Analysis by region revealed a significant negative correlation between disease duration and NFT in the SNM using graded data (Spearman's rho -0.474, p=0.026) as well as raw count data (Spearman's rho -0.384, p=0.014). There were no significant linear correlations between grade of severity and NFT in the other regions or in any regions for TA, CB or threads. There were no significant correlations between any PSP pathological lesions and age at disease onset or age at death.

Repeatability

The intra-rater SD was 0.012, implying that 95% of severity grades fell within 0.024 either side of the measured mean, and variability of grading was thus less than 2%. The repeatability within each region differed according to size of region and variability of random sampling points. The minimum intra-rater SD was 0.003 (<1% variability) in medial SN and the maximum intra-rater SD was 0.14 (14% variability) in caudate. Overall the grading scale was highly repeatable.

Tau pathology according to clinical phenotype

The overall severity of AT8 positive lesions was significantly higher in RS (median grade 1.1; SD 0.36) than PSP-P (0.5; SD 0.23; Mann-Whitney U, $p<0.001$) and PAGF (0.4; SD 0.47; Mann-Whitney U, $p<0.001$). (Figure 8.5) Mean regional severity was always highest in RS, and significantly higher than PSP-P in all areas (Mann-Whitney U, $p<0.05$) except the dorsolateral putamen (Mann-Whitney U, $p=0.15$) and STN (Mann-Whitney U, $p=0.77$) (figure 8.6). Tau pathology was significantly more severe in RS than PAGF in the parietal grey matter (Mann-Whitney U, $p=0.022$), griseum pontis (Mann-Whitney U, $p=0.003$), dentate nucleus (Mann-Whitney U, $p=0.010$) and cerebellar white matter (Mann-Whitney U, $p=0.023$). (Figure 8.6)

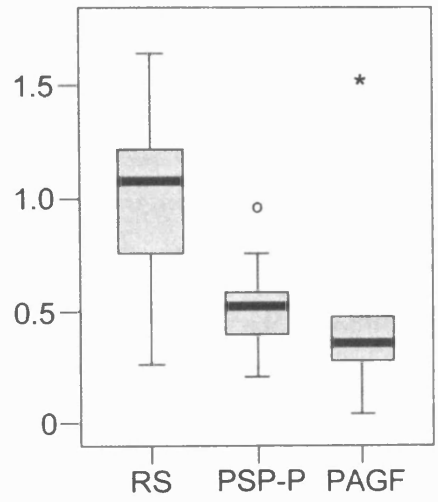


Figure 8.5 Overall tau severity according to clinical group: median, quartiles, outliers

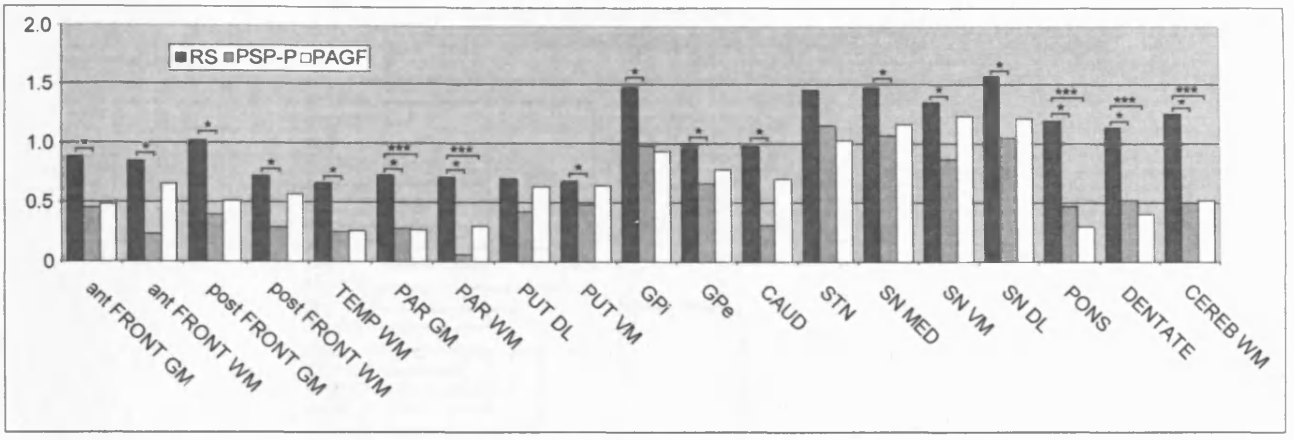


Figure 8.6 Regional tau severity according to clinical group

Patterns of tau distribution

Three clusters of cases could be identified on the distance matrix and dendrogram of the cluster combine of the cluster analysis, and five individual cases could be deemed not to belong to any cluster. (Figure 8.7) The cases in cluster 1, the largest group (group 1, n=18; 44% RS, 56% PSP-P) were characterised by relatively more tau pathology in the GPi, STN and SN, than in the cortical regions, griseum pontis and cerebellar white matter. The median overall tau grade of this group was 0.48 (interquartile range (IQR) 0.18-0.88). In the second cluster (group 2, n=4; 100% RS) cases had relatively more tau pathology in the posterior frontal grey matter, putamen, GPi, caudate, STN and SN than in the temporal white matter, dentate nucleus and cerebellar white matter. The median overall tau grade of this group was 0.86 (IQR 0.5-1.29), and was significantly higher than group 1 (Mann Whitney U, $p<0.001$). In the third cluster (group 3, n=7; 86% RS, 14% PAGF) cases were characterised by relatively more severe tau pathology in the frontal cortical regions, GPi, STN, SN as well as the griseum pontis and cerebellar white matter, and the median overall tau grade (1.14, IQR 0.68-1.63) was significantly higher than in the other groups. (Table 8.3) Median regional severity significantly differed between groups (see figure 8.8). In group 1, cortical pathology was significantly less than in groups 2 and 3. The severity of tau pathology in the STN and SN was similar between all groups but in the griseum pontis and cerebellar white matter it was more severe in group 3 than groups 1 and 2. In group 2 the caudate was significantly more severely affected than in groups 1 and 3.

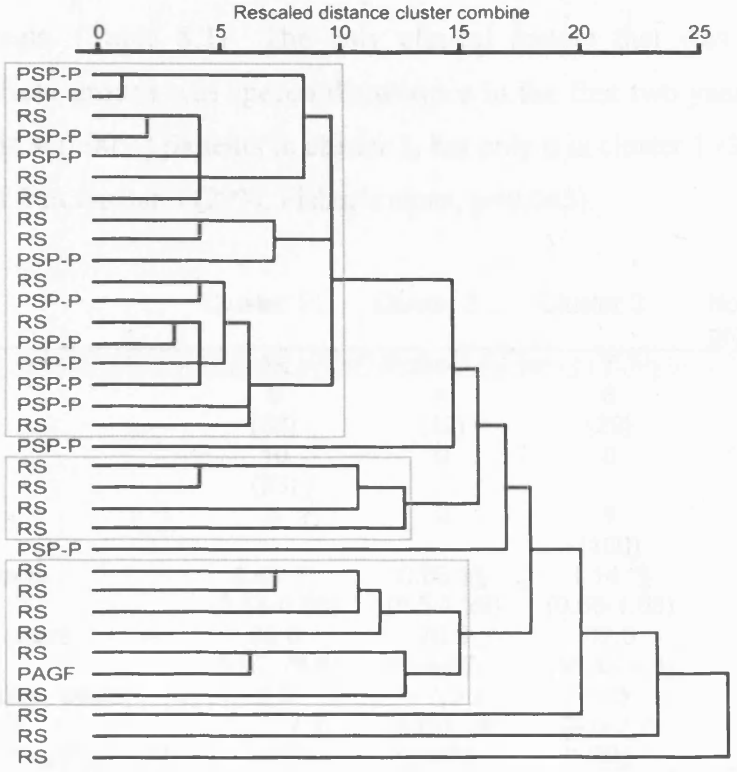


Figure 8.7 Hierarchical cluster analysis of cases: dendrogram using average linkage (between groups).

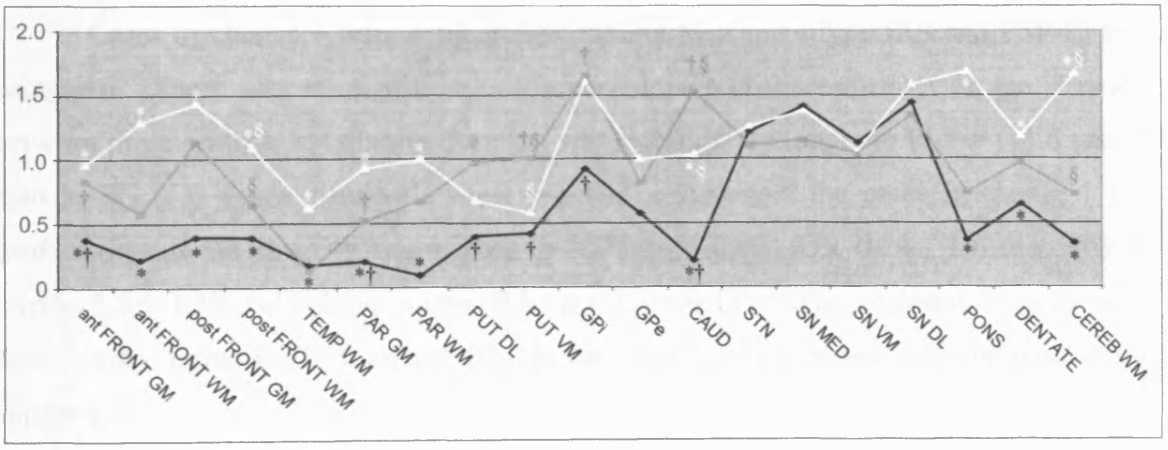


Figure 8.8 Distribution of tau pathology according to cluster: median values in cluster 1 (black) cluster 2 (grey) and cluster 3 (white); † $p<0.05$, cluster 1 vs. 2; * $p<0.05$, cluster 1 vs. 3; § $p<0.05$, cluster 2 vs. 3.

There were no significant differences in mean disease duration or age at disease onset between groups. (Table 8.3) The only clinical feature that was significantly different between these groups was speech disturbance in the first two years of disease, which was present in 4 (100%) patients in cluster 2, but only 6 in cluster 1 (33%, Fisher's exact, p=0.029) and 2 in cluster 3 (29%, Fisher's exact, p=0.045).

	Cluster 1	Cluster 2	Cluster 3	Not grouped
Number	18	4	7	5
RS	8	4	6	3
(%)	(38)	(19)	(29)	(14)
PSP-P	10	0	0	2
(%)	(83)			(17)
PAGF	0	0	1	0
(%)			(100)	
Median tau grade	0.48 †*	0.86 †§	1.14 *§	
(IQR)	(0.18-0.88)	(0.5-1.29)	(0.68-1.63)	
Age at onset, years	65.0	70.0	67.5	
(range)	(51.2-76.6)	(56.4-87.5)	(58.1-74.3)	
Disease duration, years	9.4	7.5	6.3	
(range)	(1.2-17.3)	(5.0-10.8)	(4.0-7.2)	
Early speech disturbance %	33*	100*§	29§	

Table 8.3 Demographics of cluster groups, † $p<0.05$, cluster 1 vs. 2; * $p<0.05$, cluster 1 vs. 3; § $p<0.05$, cluster 2 vs. 3

Cases in cluster 1 were separated according to clinical type (RS and PSP-P) and compared. There was no significant difference in age at disease onset or age at death between these groups, but disease duration was significantly longer in PSP-P (11.6 years) than in RS (5.6 years, Student's t-test p=0.003). Amongst the cases in cluster 1 the median overall tau severity was higher in RS (grade 0.62, IQR 0.29-1.10) than PSP-P (grade 0.37, IQR 0.17-0.69, *Mann Whitney U* p=0.12). The regional severity was significantly higher in RS than in PSP-P in the parietal white matter, GPi and medial SN (figure 8.9).

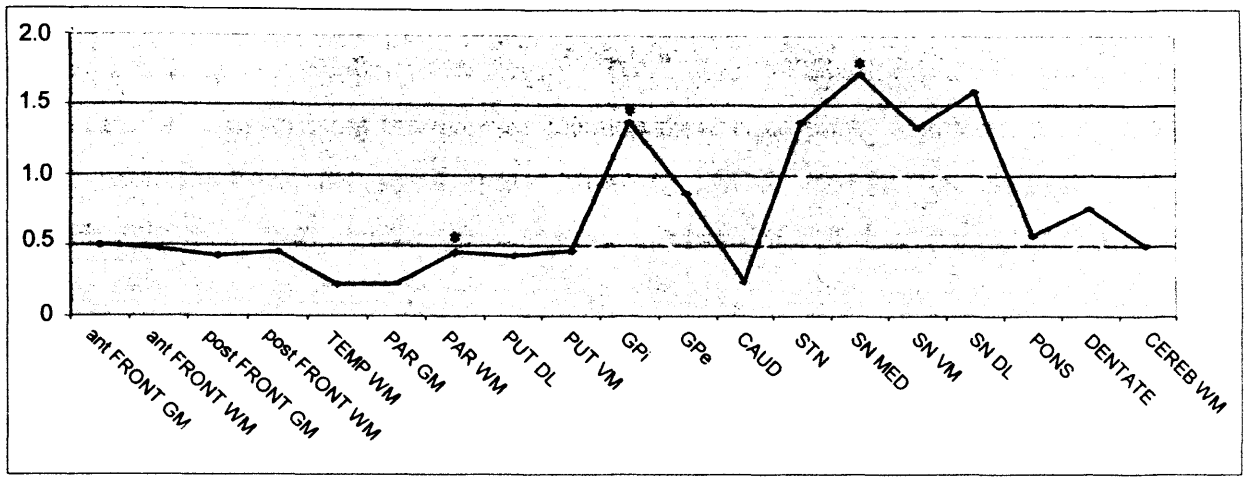


Figure 8.9 Distribution of tau pathology in cluster 1 according to clinical phenotype: median value in RS (black) and PSP-P (white); * *Mann Whitney U p<0.05*, RS vs. PSP-P

Discussion

This study confirms that PSP-P and PAGF exist as clinical manifestations of PSP-tau pathology, and are characterised by the typical pathological hallmarks of PSP. We have documented substantial variability in the distribution and severity of pathological tau accumulation in PSP and have found that co-existent additional pathological diagnoses do not differ between RS, PSP-P and PAGF, implying that variability in tau pathology is the principal factor separating these clinical syndromes.

Pathological tau accumulation was found to be most severe in RS, and its distribution throughout the brain was more widespread than in PSP-P and PAGF. The tau pathology in RS was predominantly subcortical affecting the STN, SN and GPi but also involved the cerebral cortex and was consistently more widespread and severe than in the other clinical types. In PSP-P severe tau pathology tended to be restricted to pallido-luyso-nigral structures, with less involvement of the cerebral cortices, griseum pontis, dentate nucleus and cerebellar white matter. In PAGF tau pathology was less severe than in other types, and also had a more restricted distribution than RS.

Three distinct patterns of pathological tau distribution were identified by cluster analysis. In the most frequently encountered pattern, the burden of tau accumulation was

highest in the STN, SN and GPi, and relatively spared the cortical regions, caudate, griseum pontis and cerebellar white matter. This pattern of distribution was common to RS and PSP-P, emphasising the overlap between these conditions. However, the severity of tau accumulation was significantly lower in PSP-P than in RS, suggesting the separation of these two syndromes along the spectrum of PSP-tau changes. A second pattern, only seen in RS, had more severe pathology in the caudate, putamen and posterior frontal lobes, while the third type had more severe pathology in the griseum pontis and cerebellar white matter and all cortical regions, in particular the frontal cortex.

Pathological features additional to PSP-tau lesions were present in very few patients. When Lewy body pathology coexists with PSP it is considered to represent a different disease process, which could potentially influence the clinical phenotype. (Uchikado *et al.*, 2006) Reports of previous pathological series have found coexistent Lewy body pathology in approximately 10% of patients with PSP, which is similar to age matched control brains. (Gearing *et al.*, 1994; Tsuboi *et al.*, 2001; Uchikado *et al.*, 2006) The frequency of Lewy body pathology in this series was substantially lower (2.4%). There were no significant differences in the degree of Alzheimer type pathology between the three clinical phenotypes. Co-existent argyrophilic grain disease (AGD) has been previously reported in PSP, but the frequency of this association is uncertain. (Martinez-Lage and Munoz, 1997; Tolnay *et al.*, 2002; Togo and Dickson, 2002; Tsuboi *et al.*, 2005) Tau-positive grains of considerable degree were identified in ten cases (23%) and were more frequent in PSP-P than RS, although this difference did not reach significance. In pure AGD, patients are most likely to present with memory disturbance and personality change (Tolnay *et al.*, 2002) and its coexistence with PD has been linked occasionally with ophthalmoplegia and cognitive disturbance. (Seno *et al.*, 2000) Mild ischaemic vascular pathology was present in a minority of patients (31%), and did not differ between clinical types.

No differences were found between the distribution of AT8 and RD4 antibody stains. RD4 positive NFTs, threads, CB and TA were present in all cases, whereas RD3 immunoreactive structures were absent in the material examined, which did not include entorhinal and transentorhinal cortical regions. Previous immunoblot analysis of insoluble tau from the basal pons in PSP demonstrated the presence of varying degrees of

3-repeat tau. (Gibb *et al.*, 2004) Despite this, we were unable to identify any staining using RD3, which is a sensitive and specific antibody to 3-repeat isoforms. (de Silva *et al.*, 2003) This discrepancy is possibly due to levels being below the detection threshold of RD3 or masking of the 3R-tau epitope in tissue. On an immunoblot, 3-repeat tau may be more concentrated or exposed as a result of denaturation explaining the discrepancy. (de Silva *et al.*, 2003)

The relationship between neuronal loss, tau accumulation and neuronal dysfunction in tauopathies is incompletely understood. (Daniel *et al.*, 1995; Halliday *et al.*, 2000) However, there is some evidence that the extent and severity of tau pathology reflects clinical features. Cognitive dysfunction has been associated with increasing cortical tau pathology (Braak *et al.*, 1992; Bergeron *et al.*, 1998; Bigio *et al.*, 1999; Tsuboi *et al.*, 2005) and more severe tau pathology in the nucleus raphe interpositus has been correlated with supranuclear gaze palsy. (Revesz *et al.*, 1996) In contrast to these findings there is a reduction in the density of NFT in the SN with ongoing subcortical atrophy, suggesting that in certain regions tau severity correlates weakly with neuronal loss. (Halliday *et al.*, 2000) We have also identified a significant reduction in NFT correlated with increased disease duration in this region, but not in other regions. Overall, however, our findings suggest that the distribution and severity of pathological tau is an adequate measure of regional brain dysfunction that is not consistently affected by age or disease duration, and is therefore an appropriate marker for attempting to establish clinicopathological correlations.

Clinicopathological correlations

This study confirms previous observations that the STN and SN are the most severely affected structures in PSP. (Steele *et al.*, 1964; Hauw *et al.*, 1994; Daniel *et al.*, 1995) The pattern of tau distribution outside these structures is similar to that reported by Verny and colleagues who mapped the distribution of tangle formation and neuronal loss in 10 cases. (Verny *et al.*, 1996a) One group with typical clinical signs had widespread involvement of subcortical structures, and more severe involvement of the pedunculopontine nucleus and cortex than a second group with unusual clinical features and mild involvement of the pedunculopontine nucleus and cerebral cortex. Similar to

cluster one in the present study, this group had more severe involvement of the subcortical structures in a pallido-luyso-nigral distribution. These findings suggest that there is a consistent regional vulnerability to PSP-tau pathology in the STN, SN and GP and that clinical differences are related to differences in the distribution of pathological changes outside the basal ganglia.

We found no convincing evidence that age or disease duration affected PSP-tau pathology. NFTs in the SN were the only pathological lesion inversely related to disease duration. One previous report, using semiquantitative data and a 4 point rating scale, found an inverse relationship between disease duration and oligodendroglial pathology, particularly in the globus pallidus. (Josephs *et al.*, 2006b) In the present study we applied a region and lesion specific 5 point rating scale with high intra-rater reliability, and failed to confirm this finding. This discrepancy emphasises that the regional pathological variability in PSP means that no single set of parameters for rating tau lesion severity will be sufficiently sensitive in all brain regions and that any single rating scale will be subject to substantial ceiling and floor effects.

In RS gaze palsy, dementia, postural instability and falls occur early in the disease. The postural instability in PSP is thought to be caused by lesions in a number of brain regions, and in particular the pons. (Agid *et al.*, 1987) In RS the severity of deposition of abnormal tau in the griseum pontis was similar to that in the STN and SN, implying similarities in the vulnerability of these structures to tau degeneration. In contrast, the griseum pontis was relatively less affected in PSP-P, and it is possible that early pathological changes in the caudal brainstem are important in the occurrence of early postural instability seen in RS. (Agid *et al.*, 1987) Anatomical regions believed to be involved with gaze fixation and ocular motility include the dentate nucleus and pontine base where tau pathology was most severe in RS, and again similar in severity to changes in the STN and SN. In PSP-P and PAGF these regions were relatively spared compared to the STN and SN. In previous studies brain stem, structures including the rostral interstitial nucleus of the medial longitudinal fascicle, superior colliculus, and nucleus raphe interpositus, were more severely affected in patients with supranuclear gaze palsy and had more severe pathology than those with "atypical" PSP where gaze palsies occurred late in the course of the illness or not at all. (Daniel *et al.*, 1995; Revesz

et al., 1996) The frontal cortex was consistently most severely affected in RS, which may reflect the early cognitive and neurobehavioral changes seen in this syndrome. In contrast, in PSP-P where cognitive dysfunction occurs late or not at all, the relative tau burden in cortical regions was very low.

In the early stages of PSP-P the clinical features are reminiscent of Parkinson's disease. The asymmetry at disease onset argues for less widespread pathology early and the restricted pattern of pathological involvement at death indicates that tau pathology continues to be more limited in extent and severity than in RS. The majority of patients with PSP-P have an initially good therapeutic response to levodopa medications, although secondary non–responsiveness later in the course of the illness is common. (Birdi *et al.*, 2002) The balance of pathological dysfunction in the motor loops of the basal ganglia is likely to be responsible for this difference with RS. It has been suggested that in PSP dopaminergic medications are relatively ineffective because of loss of post synaptic striatal dopamine receptors and cholinergic interneurons in the putamen. The absolute severity of tau pathology in the putamen is lowest in PSP-P, which would be in keeping with this notion. In addition, the less severe tau pathology in the cortical regions and the griseum pontis in PSP-P may represent involvement of these structures later in the course of the disease and explain the delayed appearance of falls, frontolimbic dementia and gaze palsies.

The clinical presentation of PAGF is quite distinct from RS and PSP-P and the tau burden is considerably lower than RS (see figure 8.4), particularly in the pedunculo-pontine region and parietal lobes. The small number of PAGF cases in this series limited the effect that this type had on the cluster analysis. However univariate analysis of the mean regional tau pathology suggested a pattern of predominantly posterior frontal lobe involvement together with involvement of the STN and SN. Interestingly previous SPECT studies in patients with (pure) gait initiation failure (Atchison *et al.*, 1993), which we consider to be the same clinical syndrome as PAGF, implicate the supplementary motor areas of the posterior frontal lobe. Other reports suggest that the globus pallidus is responsible for the akinesia in PAGF, but isolated, severe pallidal lesions were not seen in this series, and have not been reported in other pathologically examined cases of PSP-PAGF. In PAGF the evolution of clinical features, other than gait

disturbance, is slow compared to RS. The late evolution of gaze palsy in PAGF may be related to the relatively mild pathology in the griseum pontis and dentate nucleus, structures important in the generation of saccadic eye movements examined in this study. Clinically significant cognitive dysfunction is also rare but mild or moderate pathological involvement in the frontal cortex was a common finding, although lower than in RS.

Vascular pathology was not a significant finding in patients with PAGF. Lewy body pathology has previously been reported in a clinically identical syndrome classified as primary progressive gait freezing, however, the authors suggested that the presence of rigidity differentiated this patient from "pure akinesia". (Factor *et al.*, 2006) In the present series one patient with Lewy body pathology developed dementia and visual hallucinations in the last eight years of a 25 year disease (chapter 5). Pallidonigro-luysian atrophy (PNLA) has been reported rarely in the literature, but is consistently associated with bradykinesia and occasionally rigidity and catatonia. (Takahashi *et al.*, 1977; Kawai *et al.*, 1993; Mori *et al.*, 2001; Konishi *et al.*, 2005) Sparse neurofibrillary tangle pathology has been reported and PNLA has been defined by these minimal findings on conventional silver staining in the presence of severe gliosis and cell loss. (Contamin *et al.*, 1971) A recent report has identified more severe neuronal and glial tau-pathology in PNLA identified by the application of modern immunohistochemical techniques. (Mori *et al.*, 2001) Immunoblotting of phosphorylated tau from this case showed a predominance of 4R tau, raising the possibility that PNLA is a form of 'minimal change' PSP-tau pathology.

Neuropathological classification

Specific histological criteria have been proposed for PSP to allow for its distinction from other disorders in which neurofibrillary tangles occur. (Hauw *et al.*, 1994) The proposed NINDS-SPSP pathological criteria stipulate that a minimum number of subcortical regions need to be examined to make the pathological diagnosis in the context of a compatible clinical history. The criteria rely on the presence of numerous NFT and threads in the brainstem and basal ganglia, including two or more of these lesions per high powered field (h.p.f.) in the pallidum, STN, SN and griseum pontis, as well as one or more lesion per h.p.f. in three of the striatum, oculomotor complex,

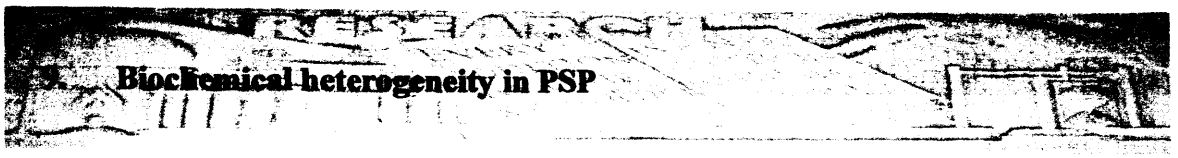
medulla and dentate nucleus. The predominance of tau pathology in this distribution in PSP is supported by our findings. (Figures 8.6 and 8.8)

Criteria for "atypical" pathological PSP have also been proposed and defined by the presence of fewer NFT and threads in the same five regions as with PSP. The criteria for atypical PSP also stipulate that the distribution of cortical NFT could be either absent or of high density in the entorhinal, prefrontal, precentral and other associative cortices. Subsequent revisions to the criteria excluded the atypical classification because of practical difficulty in reliably distinguishing it. (Litvan *et al.*, 1996b) The authors raised concern about the value and validity of an atypical pathological classification in the absence of any absolute histologic differences in clinically atypical cases (Daniel *et al.*, 1995; Litvan *et al.*, 1996b) However, the patterns of tau distribution identified by cluster analysis and the regional variability identified in the current study suggest that such a distinction is possible and may be clinically meaningful. Using these data it is possible to construct a framework for considering variability in PSP pathology. Such a framework could include the following classifications: typical PSP (RS in cluster 1), where pathology is most severe in the GP, STN, SN but also involves the griseum pontis, dentate nucleus and cerebellar white matter and cortical structures; minimal change PSP (PAGF and PSP-P in cluster 1), where pathology is restricted to the GPi, STN and SN, and relatively spares the cortex, griseum pontis and cerebellar white matter; limited cortical PSP (RS, cluster 2), where the most severe pathology exists in the GP, STN, SN and caudate nucleus, there is moderate pathology in the griseum pontis and cerebellar white matter and some cortical regions with tau lesions of moderate severity; and widespread PSP (RS, cluster 3), where there is moderately severe tau pathology within the parietal and anterior and posterior frontal cortices, griseum pontis, dentate nucleus and cerebellar white matter in addition to the GPi, STN and SN.

Conclusion

We have confirmed that PSP-P and PAGF are clinical syndromes with PSP-tau pathology, and are characterised by all of the typical pathological hallmarks of PSP. The findings of this study argue that the clinical differences between "clinically atypical" PSP (PSP-P and PAGF) and RS are underpinned by tau pathology that is less severe and less

widespread than in RS and are not due to co-existent additional pathological disease. The cluster analysis demonstrated that the distribution of tau pathology in PSP-P was similar to RS, but was consistently milder. Furthermore, only in RS was severe cortical, pontine and cerebellar white matter tau pathology present. A pathological sub-classification to incorporate these observations, including the extent and distribution of PSP-tau, should provide a plausible framework for determining further clinicopathological correlations. The factors which contribute to the pathological and clinical differences between these groups remain to be determined.



PSP and other tauopathies are characterised by neuronal, and in some cases glial, accumulation of tau. Accumulations of insoluble tau contain different ratios of the six tau isoforms. Disease specific tau isoform profiles are examined using western blot analysis of brain tissue homogenates. In the preparatory experiment two methods of tissue preparation were compared to determine the most efficient and efficacious method for examining insoluble tau in PSP. The subsequent experiment used the 'Hanger' method to examine the diversity of tau isoform profiles amongst 69 cases of pathologically diagnosed PSP. The isoform composition of insoluble tangle-tau isolated from the basal pons differed significantly between RS and PSP-P. In RS the mean ratio of 4-repeat:3-repeat tau was 2.84 and in PSP-P it was 1.63 ($p<0.003$). The differences in tau deposition provide a tantalising clue that the molecular processes the lead to neurodegeneration in PSP may vary between these two clinical phenotypes.

Introduction

In the CNS, the tau protein occurs as six isoforms ranging from 352 to 440 amino acids, with molecular weights from 45 to 65 kDa (figure 2.1). These isoforms contain either three or four repeat domains (3R-tau or 4R-tau), according to the absence or presence of 31 amino acid microtubule-binding repeat. This is the result of the alternative splicing of the tau gene (*MAPT*) exon 10, which codes for the second of four microtubule-binding repeat domains of the tau protein, the other 3 being encoded by exons 9, 11 and 12. Both 3R and 4R-tau variants are further separated according to the presence or absence of 2 different amino acid sequences encoded by *MAPT* exons 2 and 3, at the amino-terminal end, thus giving rise to the six isoforms designated 0N3R, 1N3R, 2N3R, 0N4R, 1N4R and 2N4R (0N, 1N and 2N indicating the presence of 0, 1 or 2 of the amino-terminal inserts).

Phosphorylation regulates the microtubule binding capacity of tau and probably modifies its biochemical properties by making the tau molecule longer and stiffer (Hagestedt *et al.*, 1989) Increased phosphorylation, or hyperphosphorylation, occurs in neurodegenerative disorders but it is unclear whether this occurs before or after filamentous aggregation. In AD, which is the best studied 'secondary' tauopathy, the

paired helical filaments (PHF) that form characteristic NFTs are known to be comprised of insoluble, fibrillar tau (PHF-tau). PHF-tau deposited in AD differs from normal, soluble tau by its hyperphosphorylated state, which also has the effect of reducing its electrophoretic mobility. (Flament *et al.*, 1989; Hanger *et al.*, 1991) The biochemical characterisation of these insoluble aggregates by SDS-PAGE and tau-immunoblotting reveals patterns involving all of the constituent isoforms. On western blots, AD PHF-tau runs in three major bands, corresponding to proteins at 55, 64 and 69 kDa, and a fourth minor tau species at 74 kDa. The characteristics of insoluble tau inclusions have been examined in different tauopathies and other tau isoform patterns have been identified in PiD (Delacourte *et al.*, 1996; Lieberman *et al.*, 1998; Zhukareva *et al.*, 2002), CBD (Ksiezak-Reding *et al.*, 1994), PSP (Vermersch *et al.*, 1994; Morris *et al.*, 2002a; Mochizuki *et al.*, 2003), PDC Guam (Buee-Scherrer *et al.*, 1995; Winton *et al.*, 2006), and FTDP-17. (Hutton *et al.*, 1998) Selective tau deposition in these apparently distinct clinical and pathological entities differ by the relative amounts 4R and 3R-tau. (Goedert *et al.*, 1998; Mailliot *et al.*, 1998) Results are often expressed only in terms of triplet or doublet banding patterns derived from hyperphosphorylated tau, which only gives imprecise estimations of the levels of individual tau isoforms. (Hong *et al.*, 1998; Buee and Delacourte, 1999) However, Goedert and co-workers demonstrated that dephosphorylation of PHF-tau proteins, using *Escherichia coli* alkaline phosphatase, reveals all six tau isoforms as separate bands according to their respective molecular weights, allowing for precise identification of each isoform (figure 2.1). (Goedert and Jakes, 1990)

Several recent refinements in the methodology for the isolation of insoluble pathological tau have improved the sensitivity and specificity of tau isoform detection by western blot analysis. (Gibb *et al.*, 2004) In the late 1980s Rubenstein and co-workers developed a technique using 1% N-lauroylsarcosine, sodium salt (sarcosyl) to separate insoluble PHF-tau and native, normally phosphorylated soluble tau from supernatants of brain homogenates by high speed centrifugation. (Rubenstein *et al.*, 1986) However, Hanger and co-workers showed that high–speed centrifugation in the absence of the sarcosyl solubilisation step can achieve the same results. (Hanger *et al.*, 1998) These two methods have not previously been compared in studies that use protein

dephosphorylation techniques. The greater efficiency of the Hanger method makes it preferable when large numbers of cases are being analysed. In the preparatory experiment (chapter 9.1) the Hanger method was compared to the modified Rubenstein method, using sarcosyl solubilisation to enrich the insoluble tau fraction. (Goedert *et al.*, 1992)

Tau isoform profiles in PSP

The majority of reports relating to the tau isoform profile in PSP have not used these dephosphorylation techniques. Insoluble tau from PSP has been characterised by a major doublet pattern of hyperphosphorylated tau on western blot of bands at 64 and 69 kDa and a weaker band at 74 kDa, (Flament *et al.*, 1991) containing predominantly 4R-tau. (Sergeant *et al.*, 1999) Biochemical mapping of neurofibrillary degeneration in PSP has shown that regional tau isoform composition of insoluble tau can vary, in particular in the entorhinal cortex where AD-type triplet bands have been found in cases where PSP doublet tau was found in the pre-motor and pre-frontal areas, caudate and GP. (Vermersch *et al.*, 1994; Schmidt *et al.*, 1996) This finding is explained by coexisting age related NFTs.

Morris and colleagues reported the insoluble tau isoform composition in a series of 26 cases of PSP separated according to typical or atypical clinical features. (Morris *et al.*, 2002a) Additional Alzheimer type pathological changes were present in 19 (74%). Tissue was taken predominantly from the GP and run on SDS-PAGE without dephosphorylation. A doublet pattern, described by the authors as a "normal PSP-tau protein electrophoretic pattern" was identified in 13 (50%) and the other cases demonstrated either a triplet pattern or six to eight protein bands, which appeared to migrate with the six recombinant tau bands. (Morris *et al.*, 2002a) These non-doublet banding patterns occurred more frequently in the clinically atypical group (66.7%) than the clinically typical group (27.3%), but did not correlate with the Alzheimer pathology. Definitive identification of the isoforms could not be made because the tissue homogenates were not dephosphorylated.

This tau protein heterogeneity in PSP was further investigated by a number of other authors who reported the presence of both 3R and 4R-tau isoforms in sporadic PSP,

accounting for the presence of the uncharacteristic banding patterns in 50% of Morris's cases. (Liu *et al.*, 2001; Hanger *et al.*, 2002; de Silva *et al.*, 2003) Gibb and colleagues used protein dephosphorylation to identify the individual tau isoform composition of clinically typical and atypical cases. (Gibb *et al.*, 2004) Samples from the pontine base in 20 cases were analysed using insoluble tau extracts without dephosphorylation and run on SDS-PAGE. Similar to the findings in the GP reported by Morris and colleagues, they found two broad groupings of tau immunoreactivity. The first had the typical PSP-tau doublet in 12 (60%) and the second had a more diffuse staining. In 11 cases the tissue homogenate was solubilised in guanidine and dephosphorylated using the highly efficient and reproducible method of dephosphorylation with lambda phosphatase described by Hanger. (Hanger *et al.*, 2002; Gibb *et al.*, 2004) They found marked variation between different cases of the guanidine solubilised, dephosphorylated tau. All six tau isoforms were present to some degree, and in many 1N3R was present in the same proportion as 4R isoforms. Taken together, these results showed that the banding pattern of hyperphosphorylated tau, derived from western blots of crude extracts of insoluble PSP-tau and probed with polyclonal pan-specific tau antibodies, cannot be used with any degree of certainty to identify the tau isoforms present in the PSP brain.

In the absence of a "typical" PSP-tau isoform profile, there remains the possibility that variability in the tau biochemical profile may account for some of the observed clinical or pathological heterogeneity.

9.1. Comparison of methods for tau fractionation and dephosphorylation

Aims

The aim of this preparatory experiment was to compare two different methods of preparation of brain tissue for the isolation and analysis of insoluble tau (Rubenstein method: sarcosyl separation; and Hanger method: centrifugal separation). (Rubenstein *et al.*, 1986; Hanger *et al.*, 2002)

Materials and methods

Pathological material

Frozen brain tissue from the pontine base and temporal lobe of two cases of neuropathologically confirmed PSP and the temporal lobe of one case of neuropathologically diagnosed AD was obtained from the QSBB, London, UK.

Preparation of soluble tau

Human brain tissue weighing 0.4-1.0 *g* was homogenised in 0.1M MES buffer, pH 6.5, containing 1M NaCl (homogenisation buffer) and clarified by centrifugation at 27,000 x *g* for 30 minutes at 4°C.

For the Rubenstein method, 1% (w/v) sarcosyl was added to resulting supernatant, incubated with shaking for 1 hour at room temperature and centrifuged at 100,000 x *g* for 1 hour at 4°C. The 100,000 x *g* pellet containing the insoluble tau, was washed once by reconstituting in 0.1M MES buffer, pH 6.5, containing 1M NaCl made up to 1% sarcosyl and re-centrifuged at 100,000 x *g* for 1 hour at 4°C. The resulting 100,000 x *g* pellet containing insoluble tangle-tau was retained.

For the Hanger method, the clarified supernatants from the first low-speed centrifugation were re-centrifuged at 100,000 x *g* for 1 hour at 4°C. The pellets containing the insoluble tau were washed by adding homogenisation buffer and recentrifuging at 100,000 x g for 1 hour at 4°C. After decanting the supernatant, the pellet was reconstituted in 0.1M MES buffer, pH 6.5, containing 1M NaCl but without the addition of sarcosyl at any time.

For both methods the resulting 100,000 x *g* supernatant contained soluble tau. The soluble tau was enriched further by heating for 10 minutes at 100°C and centrifuging at 15,000 x *g* for 30 minutes at 4°C. The supernatant, containing heat-stable soluble proteins, was made to 45% saturation with ammonium sulphate, maintained in ice for 15 minutes, and centrifuged at 15,000 x *g* for 30 minutes at 4°C. Precipitated proteins were re-suspended in 0.1 x vol. (relative to weight of starting brain material) 50mM Tris-HCl pH 7.5 and dialysed against the same buffer overnight at 4°C. The dialysate was clarified by centrifugation at 15,000 x *g* for 30 minutes at 4°C, and the supernatant, containing soluble heat-stable control brain tau, was retained.

Preparation of guanidine solubilised tau

The 100,000 x *g* pellets were solubilised with guanidium hydrochloride (guanidine-HCl). (Hanger *et al.*, 1998) For both the Hanger and Rubenstein methods the 100,000 x g pellet, containing insoluble tangle-tau, was solubilised in 50mM Tris-HCl, pH 7.5 containing 4M guanidine-HCl. The solution was agitated for 60 minutes at room temperature and the guanidine solubilised tau was then dialysed into 50mM Tris-HCl, pH 7.5 overnight at 4°C and centrifuged at 7000 rpm for 1 minute to remove precipitated material.

Tau dephosphorylation

Soluble tau and guanidine-solubilised tau extracted from all three cases were each dephosphorylated with lambda protein phosphatase (New England Biolabs) as previously described (Hanger *et al.*, 2002). The tau preparations in 50mM Tris-HCl, pH 7.5, were dephosphorylated with 20 U/µl lambda protein phosphatase (New England Biolabs) for 3 hours at 30°C. Reactions were stopped by the addition of 2x SDS-PAGE sample buffer and heating for 5 minutes at 100°C.

Purification of recombinant tau isoforms

The six isoforms of tau were each expressed in *Escherichia coli* BL21 cells and purified. (Mulot *et al.*, 1994).

Analysis of tau western blots

Proteins in the guanidine-solubilised brain extracts, with and without sarcosyl solubilisation, were separated on 10% (w/v) acrylamide SDS-PAGE. (Hanger *et al.*, 1998) A ladder of all six isoforms of recombinant tau and Cruz markers were run in adjacent wells. Resolved proteins were transferred onto PVDF membranes and probed with 1:20,000 TP70, a rabbit polyclonal antiserum that recognises all forms of tau. (Brion *et al.*, 1993) The immunoreactive bands were detected using standard enhanced chemi-luminescence (Amersham Pharmacia Biotech). For semi-quantitative analysis the detected bands of dephosphorylated guanidine-solubilised and soluble tau were scanned, and the density of each band was measured using 1D Image Analysis Software (Version 3.5, Kodak).

Results

The banding patterns from the temporal lobes were identical using the two methods of tissue preparation. (Figure 9.1) In both the PSP and AD brains all six tau isoforms were present in a 4R:3R ratio of 1:1 with a predominance of the lower molecular weight bands. In the pontine base of the PSP case, the Hanger method produced a higher yield of insoluble tau. In both methods the 4R bands were stronger, but 0N3R and 1N3R bands were visible only with the Hanger method. Even after prolonged exposure of the chemi-luminescence the Rubenstein method only showed a very faint 1N3R band.

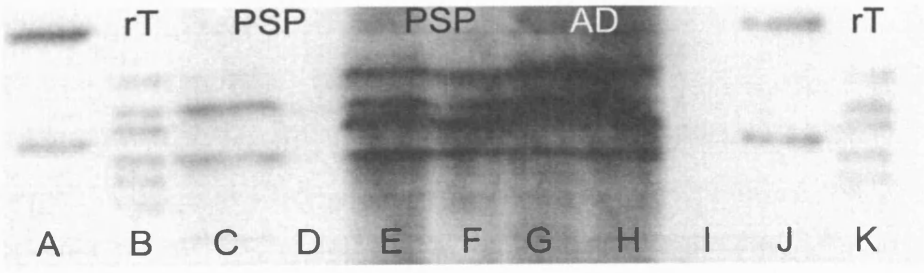


Figure 9.1 Western blots of guanidine-solubilised tau, comparing two methods tau preparation. **A** Cruz marker; **B** recombinant tau; **C** pons (Hanger method); **D** pons (Rubenstein method); **E** temporal lobe (Hanger method); **F** temporal lobe (Rubenstein method); **G** temporal lobe (Hanger method); **H** temporal lobe (Rubenstein method); **I** coloured protein ladder; **J** Cruz marker; **K** recombinant tau

Conclusion

In both methods guanidine solubilisation and dephosphorylation using lambda protein phosphatase was successful. The Hanger method, in which soluble and insoluble tau are separated using only centrifugation, was similar in efficacy to the Rubenstein method.

9.2. Biochemical heterogeneity in PSP

Aims

The aim of this experiment was to examine the heterogeneity of tau isoforms in pathological tau accumulation in PSP and to search for clinical correlations by comparing the tau isoform profiles amongst those cases clinically categorised RS and PSP-P.

Materials and methods

Pathological material

Frozen brain tissue was available from 69 cases with pathologically diagnosed PSP archived at the QSBB. In each case a portion of the pontine base, with the griseum pontis, was taken for tau isoform analysis. The pontine base was selected because it is considered to be free from Alzheimer tangles and age related tau pathology, (Parvizi *et al.*, 2001) and abnormal tau deposits are present in glial and neuronal cells of the basal pons in PSP. The composition of the insoluble tau isolated from this region was analysed by dephosphorylation of the guanidine-solubilised deposits followed by electrophoresis. The isoform profiles were compared to soluble tau that is ubiquitous in the human brain.

Precipitation of soluble and guanidine solubilised tau

Tau protein was extracted from the pontine base using the Hanger method described in chapter 9.1. (Hanger *et al.*, 1998)

Tau dephosphorylation

Soluble tau and guanidine-solubilised tau extracted from the pontine base of all 69 PSP brains, guanidine solubilised tau from the frontal lobe and pontine base of the Alzheimer brain and pontine base of the control brain was dephosphorylated with lambda protein phosphatase (New England Biolabs). (Hanger *et al.*, 2002)

Purification of recombinant tau isoforms

The six isoforms of tau were each expressed in *Escherichia coli* BL21 cells and purified as described previously. (Mulot *et al.*, 1994).

Analysis of tau on western blots

Proteins in the soluble, insoluble and guanidine-solubilised brain extracts, with and without phosphatase treatment, were separated on 10% (w/v) acrylamide SDS-PAGE (Hanger *et al.*, 1998). Resolved proteins were transferred onto PVDF membranes and probed with 1:20,000 TP70 a rabbit polyclonal antiserum that recognises all forms of tau (a generous gift from Dr Diane Hanger) (Brion *et al.*, 1993). The immunoreactive bands were detected on X-ray film using standard enhanced chemiluminescence (Amersham Pharmacia Biotech). For semi-quantitative analysis the detected bands of dephosphorylated guanidine-solubilised and soluble tau were scanned, and the density of each band was measured using 1D Image Analysis Software (Version 3.5, Kodak). For comparison the band with the highest density was given a value of 1 and the other bands were given a value equal to the ratio of their density compared to this band.

Statistical analysis of banding patterns

Cases were separated according to clinical subtype (RS or PSP-P). The relative values of the densities for each band in all cases were added and the mean in each clinical group was calculated. A Mann-Whitney U test was performed using SPSS for Windows (version 12.0.1) to check for statistical significance of the difference for each individual tau isoform and the ratio of 4R-tau to 3R-tau.

Results

Guanidine-solubilised tau extracts (i.e. the fraction containing the insoluble tau deposits) were examined in 68 cases (Figure 9.2) and soluble tau extracts were examined in 49. In one case guanidine-solubilised tau could not be detected in tissue homogenate. There was considerable heterogeneity in tau isoform profiles between individuals, including variation in all six tau isoforms. The 1N3R, 0N4R and 1N4R isoforms were most prominent in the guanidine-solubilised tau fractions, the other isoforms were present in only small quantities and were frequently not detectable at all. The soluble tau fractions were more homogenous, with all six isoforms represented in most individuals and 1N3R being present in large amounts.

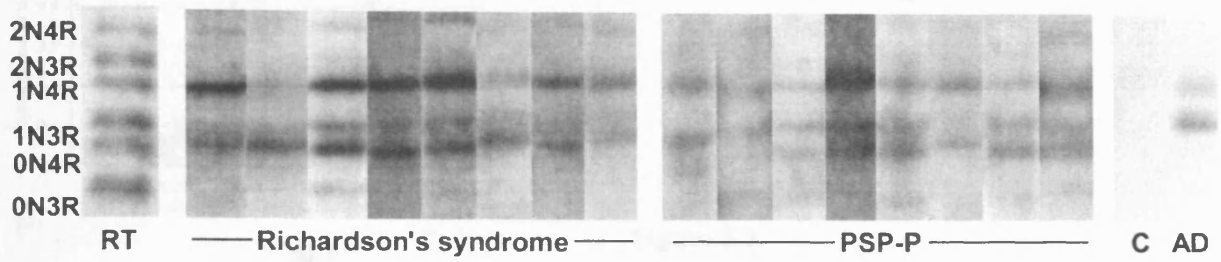


Figure 9.2 Western blots of guanidine-solubilised, dephosphorylated tau from basal pons. RT = recombinant tau; C = control; AD = Alzheimer's disease.

Results from semi-quantitative analysis of western blots were pooled according to clinical group and tau isoform. Different patterns of guanidine-solubilised tau isoforms were detected in the two clinical subgroups (figure 9.3A) but the profile of soluble tau expression did not differ (figure 9.3B). In the guanidine-solubilised fractions there were substantially more 4R-tau than 3R-tau isoforms in both groups. Pooled data from RS indicated that 1N4R and 0N4R were most prominent, 1N3R was present in smaller amounts and there were only small amounts of the other 3R-tau isoforms. In PSP-P, 1N4R and 0N4R were also most prominent but an approximately equivalent amount of 1N3R was present, significantly more so than in RS. The other 3R-tau isoforms were detected in higher amounts in PSP-P than in RS (0N3R $p=0.015$, 1N3R, $p=0.002$, 2N3R, $p=0.002$).

In conclusion, there was no difference between total guanidine-solubilised 4R-tau between the two clinical groups but there was 57% more 3R-tau in PSP-P compared to RS ($p=0.001$) (figure 9.3C). The mean 4R-tau/3R-tau ratio in RS was 2.84 (standard deviation (SD), 1.64; standard error of the mean (SEM), 0.26) and in PSP-P was 1.63 (SD, 0.88; SEM 0.19; $p<0.003$). (Figure 9.4) 3R-tau predominant deposits were identified in the guanidine-solubilised extract from the pontine base of the patient with AD. No insoluble tau was identified in the pontine base of the control patient. (Figure 9.2)

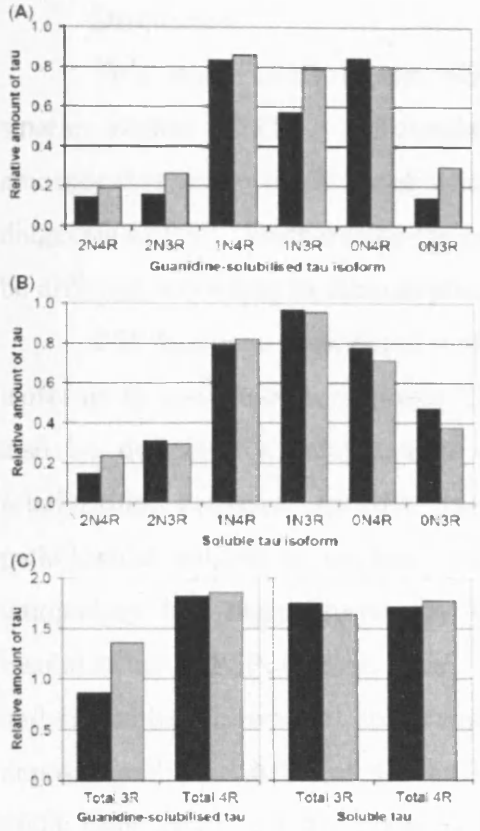


Figure 9.3

Black = RS; grey = PSP-P
(**A**) Guanidine-solubilised tau isoforms according to clinical phenotype; (**B**) soluble tau isoforms according to clinical phenotype; (**C**) total 3R-tau and 4R-tau according to clinical phenotype.

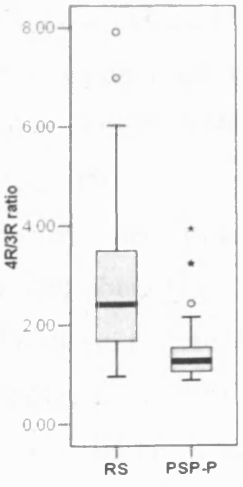


Figure 9.4
Distribution, median, upper and lower quartiles of 4R/3R tau ratios according to clinical group

Discussion

This study confirms the biochemical tau heterogeneity previously reported in smaller studies of PSP. Substantial differences exist in the tau isoform composition amongst this group of 69 cases who satisfy the operational criteria for the pathological diagnosis of PSP. Furthermore the pathological tau profile in the pontine base appears to be different according to clinical phenotype as defined by factorial analysis. (Chapter 6)

PSP has been considered a 4R-tauopathy because of the predominance of 4R-tau isoforms in insoluble tau deposits. (Buee and Delacourte, 1999) Other tauopathies can also be defined by their pattern of insoluble tau isoform aggregation though the relationship between specific isoforms and aetiopathogenesis of these clinico-pathological entities is unclear. (Buee and Delacourte, 1999) This classification of tauopathies has been blurred by the demonstration of heterogeneity in aggregated insoluble tau in PSP. (Morris *et al.*, 2002a) Analysis using specific antibodies to 3R-tau and 4R-tau has shown that, to a variable degree, 3R-tau is a component of these insoluble deposits and that different 4R-tau:3R-tau ratios exist between cases. (de Silva *et al.*, 2003; Gibb *et al.*, 2004) These differences were not related to amount of soluble tau (Gibb *et al.*, 2004) and have not previously been related to clinical phenotype. Variations in the ratio of 4R-tau to 3R-tau in PSP and other neurodegenerative conditions have now been found by a number of authors using biochemical and immunohistochemical methods. (Chambers *et al.*, 1999; Liu *et al.*, 2001; Arai *et al.*, 2001; Takanashi *et al.*, 2002; Gibb *et al.*, 2004)

There was diversity in the ratio of 4R-tau to 3R-tau, and in four cases it was less than 1. The clinical phenotypes of RS and PSP-P could be separated according to the ratio of 4R-tau to 3R-tau (figure 9.4) and the expression of soluble tau was not a factor in these differences (see figure 9.3C). It is of interest that the most "atypical" tau profiles, with relatively more 3R-tau, were from the PSP-P clinical subgroup. Another report found a three fold increase in 4R-tau/3R-tau ratio in the lentiform nucleus of all cases of PSP, without concurrent AD or neuropathological evidence of aging in other brain areas (Liu *et al.*, 2001). The cases were collected from an Alzheimer's and PSP brain bank and selection bias may have limited the number of cases with PSP-P type presentations that would, according to the current results, favour a higher 4R-tau/3R-tau ratio. The absence

of Alzheimer type pathology contributing to the banding patterns would also favour a higher 4R-tau:3R-tau ratio. The identification of cases with lower 4R-tau:3R-tau ratios in our series is unlikely to be due to coexistent Alzheimer pathology, as we extracted brain tissue from the pontine base, a region where AD NFTs do not generally occur and AD tau immunostaining has been found to be weak. (Parvizi *et al.*, 2001) Nevertheless, tau in guanidine-solubilised brain extract from the case with AD was found to have a low 4R-tau to 3R-tau ratio. In the PSP cases coexistent AD was reported in only 3 (9.1%) of the PSP-P group and 1 (1.8%) of the RS group (p=0.1) and is therefore unlikely to account for the difference in tau isoform deposition between the groups. Age-related diffuse plaques were present in 11 (19.6%) of the RS cases and in 7 (21%) of the PSP-P cases (p=0.5).

Conclusions

Considerable heterogeneity occurs in insoluble, pathological tau in PSP. The classic reports of PSP as a 4R-tau disease do not encompass the diversity that exists in a large group of clinically heterogeneous patients. The greatest divergence from the classic view of PSP-tau occurs in patients with a clinical syndrome that is substantially different from Richardson's classic description and suggests subtle differences in the ætiopathogenesis of tau accumulation in PSP-P and RS.

Previous reports have suggested that atypical clinical phenotypes of PSP occur in familial disease, and might be associated with mutations of *MAPT*. We examined the association of PSP-susceptibility tau haplotypes in pathologically diagnosed PSP, dichotomised according to clinical features (Richardson's syndrome (RS) and PSP-Parkinsonism (PSP-P)) and screened for mutations in exons 1 and 10 of *MAPT* in these patients. The association between APOE genotype and PSP was also examined. No mutations were found in 85 patients (21 PSP-P), and H1 was associated with both RS and PSP-P compared to controls. There was no association between APOE and PSP, or RS and PSP-P separately. Routine screening for *MAPT* mutations in atypical PSP is not recommended.

Introduction

Although PSP is a sporadic disorder there are a few reports of familial aggregation (David *et al.*, 1968; Mata *et al.*, 1983; Brown *et al.*, 1993), in some cases with pathological confirmation (Ohara *et al.*, 1992; Tetrud *et al.*, 1996; Ros *et al.*, 2005). In one clinical genetic study of familial PSP 44 patients from 12 pedigrees were studied and 34 had clinically typical PSP, but another 10 had unusual clinical phenotypes including features of other neurological diseases, including postural tremor, dementia, dystonia with gaze palsy and tremor and isolated gait disturbance. (Rojo *et al.*, 1999) In these patients the inheritance was consistent with a dominant pattern with reduced penetrance. The authors suggested that the rarity of familial cases may be in part due to the lack of recognition of unusual clinical phenotypes and the difficulty finding a large enough number of affected individuals in a disease of the elderly.

In contrast to FTDP-17, *MAPT* is generally not mutated in RS. (Poorkaj *et al.*, 2002; Morris *et al.*, 2002b) However there are several reports in the literature of mutations causing clinical and pathological phenotypes closely resembling typical PSP, and in some cases with the unusual clinical features predicted by Rojo and colleagues. (Rojo *et al.*, 1999) One patient reported with young onset PSP at 40 years of age, without a family history of neurological disease, had a tau exon 10 +16 (intronic) mutation. Neuropathological examination confirmed the genetic diagnosis of frontotemporal

dementia, with prolific tau deposition. (Morris *et al.*, 2003) A silent mutation S305S in tau exon 10, was identified in another patient with a history of early dementia and abnormalities of gaze, without falls or axial rigidity but with pathological characteristics of PSP. Two other affected family members who presented with clinical features unusual for PSP, including prominent early dementia with falls late in the disease and early dystonia and gaze abnormalities without dementia. (Stanford *et al.*, 2000)

Two brothers from a Spanish kindred, born from a third-degree consanguineous marriage, developed dementia and supranuclear gaze palsy in the fourth decade and died within 5 years of disease onset. A homozygous deletion at codon 296 of *MAPT* was identified in one of the affected siblings. (Pastor *et al.*, 2001) Among the heterozygous carriers, two members with PD were identified, but none of the heterozygotes developed PSP or atypical Parkinsonism. In another report of familial autosomal recessive PSP the clinical phenotype was more typical, but pathological examination revealed prominent neurofibrillary involvement of the limbic system. (Ohara *et al.*, 1992)

One other area of genetic association has been reported in a large Spanish family with a PSP phenotype linked to a locus at 1q31.1. (Ros *et al.*, 2005) Typical pathological changes were reported in one member of this family.

Mutations of MAPT cause FTDP-17

To date more than 30 different pathogenic *MAPT* mutations have been described in more than 100 families with FTDP-17. These include missense mutations, silent mutations, in-frame single codon deletions and intronic mutations. (Rademakers *et al.*, 2004) Most mutations are clustered in exons 9-13 that encode the microtubule binding domains (see chapter 2). Depending on the type and location of the *MAPT* mutation, different pathogenic mechanisms are involved. (Hutton, 2001) All mutations in intron 10 and the majority of mutations in exon 10 disrupt the balance of exon 10 splicing, altering the ratio of 3R and 4R-tau protein. Most mutations result in increased 4R-tau, though in some cases 3R-tau is increased. In cases where the clinical picture is predominantly that of early frontal lobar dementia, even if ophthalmoplegia develops, FTDP-17 is the preferred nomenclature. (Morris *et al.*, 2003) However, in cases with mutations of *MAPT*

but the clinical picture and pathology are typical for Richardson's syndrome, familial PSP is preferred. (Stanford *et al.*, 2000)

Mutations of *MAPT* in sporadic PSP

Sequence analysis of *MAPT* in a series of 96 subjects with clinically diagnosed PSP identified one patient with a missense mutation in intron 1 of the tau gene, who was later found to have pathological changes characteristic of PSP. (Poorkaj *et al.*, 2002) This mutation was not found in the other PSP subjects or in 198 additional controls. In contrast to FTDP-17, *MAPT* mutations in exons 9, 11, 12 and 13 have not been identified in any patients with a clinical or pathological diagnosis of PSP.

Genetic association of extended haplotypes at 17q21 with PSP

In 1997 Conrad and colleagues described the first evidence that there may be a genetic predisposition to PSP involving *MAPT*. (Conrad *et al.*, 1997) They found that the a_0 allele and a_0/a_0 genotype was significantly overrepresented in patients with PSP compared to normal controls. The allelic association of *MAPT* with PSP has been extended since then, and is known to cover a region of ~1.8 million base-pairs. (Pittman *et al.*, 2004) *MAPT* is nested within the centre of the high linkage disequilibrium block. Other genes identified in this region include *saitohin*, *corticotrophin releasing hormone receptor 1* and *N-ethylmaleimide sensitive factor*.

The frequency of the extended H1 haplotype in Caucasian control populations is 77% - 80%, but in PSP the allele frequency is increased to more than 93%. The odds ratio for the H1H1 susceptibility genotype in PSP was found to be 4.08 (95 % confidence interval 1.89 to 8.79). (Baker *et al.*, 1999) While the increased odds ratio for H1H1 is significant, this genotype is neither necessary nor sufficient to cause PSP. In addition there is some evidence suggesting that the H2 allele is protective. (Pittman *et al.*, 2005) No associations between genotype and pathological phenotype have been identified. In one study tau biochemistry and neuropathology was examined in 25 patients with pathologically diagnosed PSP. The H1H1 haplotype was not found to influence tau burden or tau isoform composition. (Liu *et al.*, 2001)

A family of H1-derived sub-haplotypes have now been identified. (Pittman *et al.*, 2005) A single specific variant of H1, designated the H1c haplotype, was over-represented in PSP and, importantly, other common H1 derived haplotypes showed no association with the disease. The H2 haplotype was shown to be under-represented in PSP reinforcing its probable protective role (odds ratio 0.215, 95% confidence interval 0.099 to 0.466). These findings imply that underlying variations in *MAPT* on the extended H1 background are genetic risk factors for PSP.

ApoE in PSP

Apolipoprotein E ε 4 (APOE ε4) is a widely recognized genetic risk factor for AD, (Mayeux *et al.*, 1998; Tsuang *et al.*, 1999) affecting the rate and extent of amyloid deposition (Zubenko *et al.*, 1994; Gomez-Isla *et al.*, 1996; Hyman *et al.*, 1996; Berg *et al.*, 1998; Chen *et al.*, 1999) with less consistent influence on neurofibrillary degeneration. (Ghebremedhin *et al.*, 1998; Ohm *et al.*, 1999; Mann *et al.*, 2001) There is little evidence to suggest that APOE ε4 has a similar effect on PSP. Several small studies, usually based on relatively few cases and often including exclusively clinically diagnosed cases or a mixture of clinically and pathologically diagnosed cases, have addressed the role of APOE ε4 in PSP. (Tabaton *et al.*, 1995; Schneider *et al.*, 1995; Anouti *et al.*, 1996; Litvan and Saunders, 1998; Morris *et al.*, 2000; Morris *et al.*, 2001) None of these studies has found a strong relationship between APOE ε4 and PSP.

Summary

The identification of genetic susceptibility in PSP and the similarities between patients with *MAPT* mutations and sporadic PSP raises the possibility that genetic factors account for differences between RS, PSP-P and PAGF. Furthermore, clinical genetic studies have suggested that atypical clinical syndromes occur more frequently in relatives of patients with PSP. To investigate the impact of known genetic factors on RS, PSP-P and PAGF, DNA from the group of pathologically diagnosed PSP archived at the QSBB was tested for mutations in *MAPT* and association studies were performed for tau haplotype and APOE genotype.

10.1. MAPT gene mutations in PSP

Aims

The aim of this study was to determine if *MAPT* mutations in exon 1 and 10 influence the clinical phenotype in patients with pathologically diagnosed PSP.

Methods

DNA from 79 patients archived at the QSBB with a pathological diagnosis of PSP was studied. There was no family history of neurological disease in 77, but in one there was a first degree relative with PSP and in another there was a family history of early onset AD. Patients were clinically categorised as RS, PSP-P or PAGF (chapter 6).

Tau exons (1 and 10) were amplified from genomic DNA from individuals with primers designed to flanking intronic sequences. (Andreadis *et al.*, 1992; Hutton *et al.*, 1998; Poorkaj *et al.*, 1998) Twenty-five nanograms of DNA were used in 40µl reaction mixture containing 20pmol of each primer, 0.2mM dNTPs, 1 U Taq polymerase (Qiagen, Crawley, West Sussex). Conditions were 35 cycles of 94°C for 30s, 58°C to 48°C touch down annealing for 30s, and 72°C for 45 s with a final extension of 72°C for 10 min.

The PCR product was cleaned up using 2 µl ExoSapIT (USB Amersham, Uppsala, Sweden) for 40 min at 37°C followed by 15 min at 85°C. The sequencing reaction was carried out using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The sequencing reaction was performed using forward and reverse primers separately with the following PCR conditions: 96°C for 1 min; 27 cycles of 96°C for 16 s, 54°C for 5 s and 60°C for 4 min. The sequencing products were precipitated with isopropanol, resuspended in 25 µl of water and loaded onto an ABI PRISM 3100 Genetic analyser (Applied Biosystems). The original data were analysed using Sequencing Analysis 3.7 software (Applied Biosystems) for base calling. The obtained sequences were aligned using BioEdit 7.0 software (Ibis Pharmaceuticals, Carlsbad, USA).

Results

DNA was extracted from 79 patients (55 men) with a pathological diagnosis of PSP. The mean age of disease onset was 65.2 years (range 46-87), mean age at death was 72.7 years (range 57-90) and mean disease duration was 7.6 years (range 1.2-20.9). Fifty-two patients were classified as RS, 19 as PSP-P and two as PAGF. In six patients clinical information was insufficient to assign a clinical phenotype. No mutations were identified in exons 1 or 10 of *MAPT* in any patient.

Conclusions

Mutations in exons 1 and 10 of *MAPT* are not responsible for the clinical differences observed in this group of pathologically diagnosed PSP, including patients with 'atypical' PSP phenotypes such as PSP-P and PAGF.

10.2. Tau haplotype analysis in PSP

Aims

The aim of this study was to examine the associations between tau haplotype and PSP clinical, biochemical and pathological subtype.

Methods

DNA from 85 patients archived at the QSBB with a pathological diagnosis of PSP was studied. (Baker *et al.*, 1999) Patients were clinically categorised as RS, PSP-P or PAGF (chapter 6).

Case-control allelic and genotypic association was calculated statistically in CLUMP software. (Sham and Curtis, 1995) The *p* values were derived by standard Pearson's χ^2 tests except in cases where cell counts in the contingency tables were less than 5. When cell counts were less than 5, p-values were determined empirically by 10,000 simulations; the program uses a Monte Carlo approach that performs repeated simulations to generate random tables having the same marginal totals as the one under consideration and counting the number of times that a χ^2 value associated with the actual table is achieved by the randomly generated tables.

Cases were separated according to tau genotype. Pathological severities, as described in chapter 8, and insoluble-tau isoform profiles, as described in chapter 9, were compared between these groups using χ^2 or Mann Whitney U tests as appropriate.

Results

The H1H1 genotype was significantly associated with PSP, when compared to controls (OR 5.6, 95%CI 3.7-8.5, $p<0.001$). The association was different between the two clinical subtypes (RS OR 13.2, 95% CI 3.0-57.2, $p<0.001$; PSP-P OR 4.5; 95%CI 1.3-16.0, $p=0.018$). In total 4 patients (5%) were heterozygous for the H2 allele and its overall frequency was 3.6%. The frequency of the minor allele was different between the groups, 2.8% in RS and 5.2% in PSP-P ($p=0.108$). The frequency of the H2 allele in both clinical groups was significantly less than in an unrelated control population (controls, 7.0%, $p<0.001$). There was no significant difference in the clinical features of carriers of

the H2 allele. The single patient who was homozygous for H2H2 was classified as PSP-P, and shared characteristics with that group.

There were no significant differences in guanidine-solubilised tau isoform profile or pathological tau distribution or severity between groups separated according to genotype or the presence of an H2 allele.

Conclusions

An association between *MAPT* (dys)-function and PSP has been confirmed by the OR of 5.6 for the H1H1 genotype for PSP in this study. Morris and colleagues reported that patients homozygous for H1 are more likely to present with clinically 'typical' PSP, and in their small group of 'atypical' PSP there was a higher frequency of the H2 allele. (Morris *et al.*, 2002a) We found a difference of H2 allele frequency between the two clinical subgroups, but the difference was not significant. The effect of the H1H1 PSP susceptibility genotype was stronger in RS than in PSP-P (OR 13.2 versus 4.5). The difference in genotype frequencies between the clinical subgroups was not significant. There was no correlation between genotype and tangle-tau isoform profile or pathological tau distribution or severity.

10.3. Apolipoprotein E

Aims

The aim of this study was to investigate the APOE genotype in a group of clinically well characterised patients with PSP and to examine for associations between genotype, clinical phenotype, biochemical profile and pathological profile.

Methods

In 72 patients archived at QSBB the APOE genotype was determined using a restriction digest assay as previously described. (Saunders *et al.*, 1993) Case-control allelic and genotypic association was calculated an analysed as described in chapter 10.2.

Results

Results for 68 patients were available. ApoE allelic and genotypic distributions were examined and were compared to 134 pathologically normal controls. Tables 10.1 and 10.2 show the genotype distributions and allelic distributions respectively. There were no significant differences between PSP and controls or between RS and PSP-P.

Conclusion

ApoE genotype does not appear to influence clinical phenotype or biochemical or pathological features in PSP.

	Genotype					
	2.2	2.3	2.4	3.3	3.4	4.4
PSP	1(1.4)	11 (16)	2 (3)	43 (63)	10 (15)	1 (1.4)
RS	1 (3)	4 (13)	1 (3)	22 (69)	4 (13)	0
PSP-P	0	3 (16)	0	13 (72)	1 (6)	1 (6)
Controls	0	20 (15)	5 (4)	78 (58)	30 (22)	1 (1)

Table 10.1 ApoE genotype, number (percentage)

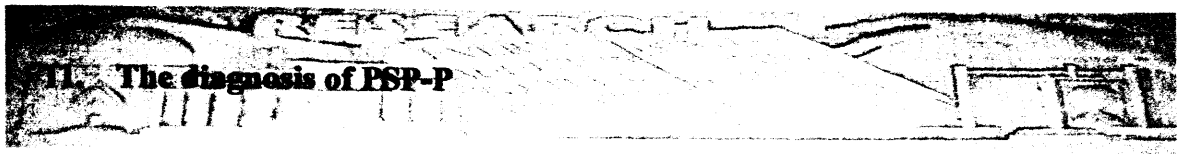
	Allele		
	2	3	4
PSP	15 (11)	107 (79)	14 (10)
RS	6 (11)	52 (81)	5 (8)
PSP-P	3 (8)	30 (84)	3 (8)
Controls	25 (9)	206 (77)	37 (14)

Table 10.2 ApoE alleles, number (percentage)

Overall conclusions

The genetic risk factors that have been identified in PSP are present in both classic PSP (RS) and atypical PSP (PSP-P, PAGF). The associations found were stronger in RS than in PSP-P, but a larger number of PSP-P patients is required to determine if there is a statistically significant difference. Mutations in *MAPT* and APOE genotype do not appear to contribute to the clinical, biochemical or pathological differences between RS, PSP-P and PAGF.

Routine screening for mutations in *MAPT* in patients with sporadic PSP or atypical Parkinsonism with clinical features of PSP-P and PAGF is unlikely to yield a high number of abnormalities. Historically, *MAPT* mutations have been identified in patients with PSP who have an early age at onset with predominant early cognitive dysfunction or a family history of neurological disease. (Delisle *et al.*, 1999; Stanford *et al.*, 2000; Morris *et al.*, 2003)



The criteria for diagnosing PD do not have sufficient specificity to exclude many cases of PSP-P. A number of clinical features have been proposed to separate PSP from PD. Olfactory function testing (University of Pennsylvania Smell Identification Test), a visual hallucination inventory (Queen Square Visual hallucination Inventory) and a modified, simple protocol testing the startle response were used to test a series of patients clinically diagnosed with PSP, PD, MSA and other bradykinetic rigid syndromes. Using case notes from the QSBB, visual hallucinations and falls were assessed retrospectively in pathologically diagnosed cases. In addition clinical features were compared between patients with pathologically diagnosed PSP, PD, MSA and VP. Visual hallucinations rarely occurred in clinically diagnosed PSP, but were frequent in PD. Olfactory function was better preserved in PSP than PD and a score below the 11th percentile for age was not found in PSP. The auditory startle response was absent or reduced in all patients with PSP. The auditory blink response was abnormal in RS but not in PSP-P, suggesting less extensive PSP-tau pathology in PSP-P. Retrospective analysis in pathologically diagnosed patients confirmed the clinical specificity of visual hallucinations for PD, and their rarity in PSP. Falls occurred commonly in all bradykinetic rigid syndromes, but were significantly earlier in both RS and PSP-P than in PD. Fractures were more common in PSP than PD or MSA. Clinical features most specific for PD and MSA, when compared with PSP, were drug induced dyskinesias, autonomic failure and visual hallucinations. PSP can be identified by the absence of these features and the presence of falls within 6 years of disease onset, UPSIT scores above the 12th percentile for gender and age, and abnormalities in auditory startle response.

Introduction

RS has been recognised with increasing accuracy since the mid 1960s. (Steele, 1975; Hughes *et al.*, 2002) The classic phenotype of early falls (often backwards) and postural instability, ophthalmoplegia, pseudobulbar palsy and a dysexecutive syndrome is highly characteristic. The accuracy of clinical diagnosis in those with PSP-P is significantly less than RS and therefore the greatest challenge faced by neurologists in identifying patients with underlying PSP-tau pathology will be to differentiate PSP-P from PD, MSA and vascular PD. The diagnostic criteria for these other conditions have previously been reported and will be considered separately in the context of the 877 cases from the QSBB that have been reviewed for this project.

UKPDSBB Diagnostic Criteria

In 1988 the UKPDSBB proposed a set of clinical diagnostic criteria for PD using clinical details extracted from the records of a group of pathologically diagnosed cases.(Appendix, Gibb and Lees, 1988) In later, carefully studied pathological series the accuracy of these diagnostic criteria was found to be up to 90%. (Hughes *et al.*, 1992a; Hughes *et al.*, 2001) In 100 consecutive cases of clinically diagnosed PD collected at the UKPDSBB the positive predictive value (PPV) and sensitivities of these criteria was found to be around 92%, and may even be higher in the hands of movement disorders specialists. (Hughes *et al.*, 2001; Hughes *et al.*, 2002) The inherent bias in this series, potentially favouring difficult to diagnose and extensively investigated cases, suggests that this is an underestimation of the predictive value of the criteria. (Maraganore *et al.*, 1999; Hughes *et al.*, 2001) PD is by far the most common cause of a progressive, bradykinetic rigid syndrome in the community. It is 25 times more prevalent than PSP (Schrag *et al.*, 1999; Nath *et al.*, 2001; Porter *et al.*, 2006) and MSA (Schrag *et al.*, 1999; Vanacore, 2005), the two conditions that it is most frequently confused with in specialist clinics. (Hughes *et al.*, 2001) The high prevalence of PD and relatively low sensitivity of the diagnostic criteria (in relation to prevalence) makes the identification of non-PD bradykinetic rigid syndromes by a negative diagnosis of PD unreliable. That is, the number of false negative diagnoses of PD will exceed the number of true negative diagnoses including PSP and MSA. The specificity of these criteria has not been calculated, but unless it approaches 100% then a positive diagnosis of PD could not absolutely exclude PSP-P. Consequently a diagnosis of PD using UKPDSBB criteria would not exclude a proportion, potentially a large proportion, of PSP-P cases.

Consensus criteria for the diagnosis of MSA

In the QSBB database 78 patients with a clinical diagnosis of MSA before death were identified. The PPV of this group's last clinical diagnosis was 81%; with 15 cases being incorrectly diagnosed MSA, including two with a pathological diagnosis of PSP. Of these two cases, one had been classified as PSP-P and the other as RS using the factorial analysis in chapter 6.1. The sensitivity of the clinical diagnosis of MSA was

68%, as 23 cases were misdiagnosed clinically as PD and seven as PSP. A consensus statement on the diagnosis of MSA was published in 1999, after many of the final diagnoses of the QSBB series had been made.(Appendix, Gilman *et al.*, 1999) These criteria were then validated in a retrospective, neuropathologically confirmed series at the QSBB. (Osaki *et al.*, 2002) As a diagnostic aid early in the disease, the strictest category "probable MSA", had sensitivity of 28%, but late in the disease the sensitivity was 63% and PPV 91%. In that study, amongst the 59 cases diagnosed with MSA, one (1.7%) had a pathological diagnosis of PSP. The misdiagnosis of PSP as MSA reflects the overlap in clinical features even using the most stringent diagnostic criteria. The consensus criteria probably have a better PPV than the final clinical diagnosis in the QSBB series, although different methodologies make direct comparisons impossible. However, the relatively high PPV at the end of disease makes it unlikely that many patients would be misdiagnosed as PSP when these criteria are applied. (Osaki *et al.*, 2002)

Vascular PD criteria

Atypical "lower body" Parkinsonism with striking 'gait apraxia' occurs with subcortical white matter lesions due to small vessel disease. However, a clinical diagnosis of VP is still difficult to make as both lacunar infarction of the basal ganglia and leukoaraiosis occur very frequently in elderly people who do not have Parkinsonism. (Zijlmans *et al.*, 2004a) Zijlmans has proposed diagnostic criteria for VP, but these have not yet been pathologically or prospectively validated. (Appendix, Zijlmans *et al.*, 2004a) One community-based survey identified a prevalence of VP about 7 times less than PD in elderly patients. (Barbosa *et al.*, 2006) In the QSBB archives 19 cases of Parkinsonism were identified in which only vascular pathology could be identified on histological examination. Of these only two were identified in life as VP, and the other 15 were misdiagnosed as PD or MSA (two cases). VP was not misdiagnosed as PSP and pathologically diagnosed PSP was not clinically diagnosed as VP. Given that the proposed diagnostic criteria include bilateral symptoms, the presence of an early shuffling gait and early cognitive dysfunction, a misdiagnosis of VP remains a possibility. (Zijlmans *et al.*, 2004a) Furthermore cases closely resembling PSP have been

reported due to vascular disease. (Tanner *et al.*, 1987; Dubinsky and Jankovic, 1987; Josephs *et al.*, 2002)

Diagnosing PSP-P

Historically the clinical diagnostic accuracy in PSP is lower than for PD, and patients with PSP-P appear to account for the majority of misdiagnoses. (Chapter 6) Together with estimates using the current operational diagnostic criteria and data from the QSBB, the greatest diagnostic dilemma appears to be between PD and PSP-P. The UKPDSBB criteria for PD do not have sufficient specificity to exclude many of these cases of PSP-P. Therefore a number of clinical features have been identified that have been proposed to separate PSP from other bradykinetic rigid syndromes, and have been predicted to increase the specificity of diagnostic criteria for PD and PSP. The clinical usefulness of these factors was explored, with a particular emphasis on clinical features that are easily assessed in the clinic. Olfactory function testing, a visual hallucination inventory and a modified, simple protocol testing the startle response were used in clinically diagnosed patients and visual hallucinations and falls were assessed retrospectively in pathologically diagnosed cases.

11.1. Diagnostic experiments in clinically diagnosed patients

11.1.1. A visual hallucination inventory that differentiates Parkinson's disease from atypical Parkinsonism

Background

Visual hallucinations (VH) and disturbances of visual perception occur in PD and are a major diagnostic feature of DLB. In PD the prevalence of VH has been reported to be between 6% and 60%. (Cummings, 1991; Diederich *et al.*, 2005) In cases with Lewy body pathology, VH are thought to be secondary to pathology in the basolateral nucleus of the amygdala, parahippocampus and associated with α-synuclein pathology in the ventral temporal and frontal cortex. (Harding *et al.*, 2002a; Harding *et al.*, 2002b; Liang *et al.*, 2005) The time from disease onset to first VH has been related to density of Lewy bodies (LB) in the parahippocampal and inferior temporal cortices. (Harding *et al.*, 2002a) The presence of VH is helpful in differentiating DLB from frontotemporal dementias and PSP, but not from AD. (Lopez *et al.*, 1999) This has led to the suggestion that VH might be helpful in the differential diagnosis of PD from atypical Parkinsonism. Patients with unclassifiable Parkinsonism make-up around 5% of patients in movement disorders specialist clinics, and most often the differential diagnosis lies between PD, MSA and PSP. (Katzenschlager *et al.*, 2003) By incorporating VH into the UKPDSBB operational clinical diagnostic criteria for PD the number of unclassifiable cases of Parkinsonism may be significantly reduced. Existing validated questionnaires that are used to screen for VH in PD, including the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn *et al.*, 1987) and the Neuropsychiatric Inventory (NPI) (Cummings *et al.*, 1994), probably underestimate the frequency of VH in PD. (Fenelon *et al.*, 2000)

Aims

A simple and short inventory capable of rapidly screening for VH, including minor forms, has been developed to determine the incidence of VH in an outpatient population with bradykinetic rigid syndromes. Its use in differentiating PD from PSP has been examined.

Methods

Developing the Queen Square Visual Hallucination Inventory

On the basis of Fenelon's description of VH in PD the Queen Square Visual Hallucination Inventory (QSVHI) was developed to determine the presence of minor and major visual hallucinations and illusions (appendix). (Fenelon *et al.*, 2000) The questionnaire was designed to be read verbatim to patients in the context of a busy outpatient clinic. Question A is a general screening question asking about VH or unusual visual perceptions over the previous three months or earlier. Question B asks specifically for minor hallucinations: 'presence' hallucinations (the vivid sensation of the presence of somebody either in the room, or less often, behind him or her-extracampine hallucinations); 'passage' hallucinations (brief visions of a person, animal or object moving across the field of vision); and illusions (visual misinterpretation). (Fenelon *et al.*, 2000) Question C asks if formed VH (consisting of people, animals or objects) were perceived and if they occurred in isolation or were associated with auditory hallucinations. Question D asks about isolated auditory hallucinations (sounds of people voices, music or other noises). The duration of all hallucinations was recorded. The physician was asked in question E to rate whether or not the hallucinations were likely to be related only to medications (VH started with initiation of medication and/or permanently disappeared on withdrawal of offending medication) or if the hallucinations occurred in the context of delirium.

Recorded data

The QSVHI was tested in three movement disorders clinics in London (National Hospital for Neurology and Neurosurgery and University College London Hospital). The study was approved by the local research ethics committee. The responses of consecutive patient's responses were noted. Routine clinical assessment including motor function was assessed in the 'on' state using the Unified Parkinson's Disease Rating Scale (UPDRS) activities of daily living and motor subscales and the modified Hoehn and Yahr staging. Cognitive function was assessed by Folstein's mini-mental state examination (MMSE). (Folstein *et al.*, 1975) Medication history, including antiparkinsonian treatments, antipsychotic treatment and antidepressant therapy was recorded. Dose and

duration of treatment was recorded and calculation of a daily L-dopa equivalent unit (LEU) dose was based on theoretical equivalence to L-dopa (Graham *et al.*, 1997) as follows: bromocriptine (mg) x 10, cabergoline (mg) x 67, ropinirole (mg) x 25, pramipexole (mg) x 100, pergolide (mg) x 100, apomorphine (mg) x 8 as described previously. (Evans *et al.*, 2004) The presence of depression, use of glasses and ocular pathology, including cataracts, retinal pathology and glaucoma was recorded.

Patients

One hundred and eighty-one patients were included. One hundred and fifteen (63.5%) patients met the UKPDSBB clinical diagnostic criteria for PD (Gibb and Lees, 1988), five (2.8%) had DLB (McKeith *et al.*, 1996), 23 (12.7%) met the NINDS-SPSP criteria for possible or probable PSP (3 PSP-P and 19 RS) (Litvan *et al.*, 1996a), 9 (5%) met the consensus criteria for MSA (3 MSA-P and 6 MSA-C) (Gilman *et al.*, 1999), five (2.8%) had probable VP (Zijlmans *et al.*, 2004a), one had CBD, two had Parkinsonism and an associated +29 tau mutation, one had a mitochondrial cytopathy, one had orthostatic tremor and in 19 (9.5%) a definitive diagnosis had not been reached. This group of patients with unclassified or undetermined Parkinsonism (UP) had either not satisfied any criteria for diagnosis or had overlapping features suggestive of more than one nosological entity. Fifteen disease-control patients with non-neurodegenerative neurological conditions selected from a different clinic (idiopathic cervical dystonia, 7; hemifacial spasm, 1; dopa responsive dystonia, 1; essential tremor, 6), and 14 control subjects, without neurological disease, were also included after written informed consent was obtained. A pathological diagnosis of CBD and Lewy body PD was made in one case which had been clinically diagnosed as CBD. Two patients with MSA and one with RS were pathologically confirmed.

VH were defined as being present if an affirmative response was given to questions A, B or C in the QSVHI. To determine the sensitivity and specificity of VH data from patients with a definitive diagnosis in the movement disorders clinic were used in a 2 x 2 table as illustrated below. This was also calculated for PD vs. PSP. For these calculations the pathologically diagnosed case and the tau+29 cases were included in the

PD group, but DLB cases were excluded because of the arbitrary clinical definition which included the presence of VH.

	PD present	PD absent
VH present	a	b
VH absent	c	d

Sensitivity = a/a+c
Specificity = d/b+d
PPV = a/a+b
NPV = d/c+d

Results

The mean age of all patients was 66.8 years old (range 20-100; SD 11.7 years), 63% were men, and mean disease duration was 9.3 years (0.3-55; SD 95.5). In PD the mean age was 65 and in PSP the mean age was 68.2 years (table 11.1). Amongst the UP patients two had early cognitive impairment and poor response to levodopa (diagnosis DLB vs. PSP), seven had a poor response to levodopa and no rest tremor (diagnosis PD vs. PSP vs. MSA), three had early autonomic symptoms, one of whom had eye movement abnormalities (PD vs. MSA vs. PSP), five had vascular risk factors, vascular changes on imaging not involving basal ganglia structures and poor response to levodopa or absence of rest tremor (PD vs. VP), and two had young onset Parkinsonism where metabolic and genetic causes had not been excluded.

There was no significant difference in frequency of ocular pathology, use of reading aids or depression between PD and the other main diagnostic groups. In PD, L-dopa and dopamine agonists were used more frequently than in the patients without PD. Compared to PSP there was no significant difference in the use of amantadine or anticholinergic medications. MMSE and Hoehn and Yahr scores indicated more severe abnormalities in PSP than PD, but UPDRS motor and ADL scores were comparable.

	PD	PSP	MSA	VP	DLB	UP	Disease control	Control
Number	115	22	9	5	6	19	15	14
Age (range) *yrs*	65 (35-85)	68.2 (58-78)	60.5 (53-69)	74.8* (62-92)	74.2 (64-80)	66.6 (20-82)	63.4 (50-87)	62.6 (42-76)
Disease duration *yrs* (range)	10.0 (1-27)	4.8* (2-15)	6.7 (2-13)	6.6 (3-13)	6.9 (3-17)	7.9 (0.3-22)	20.8* (3-55)	-
Men (%)	70	59	33†	20†	83	53	53	50
Spectacles (%)	15	14	14	20	17	11	80†	62†
Ocular pathology (%)	5	9	0	20	17	12	7	0
Depression (%)	31	27	11	20	17	18	7	NR
Medications								
L-dopa	82	52†	44†	60	83	61†	7†	0†
DA	60	10†	22†	0†	0†	28†	0†	0†
Antichol	5	5	0	20	0	6	7	0
Amantadine	41	32	44	40	17	6†	0†	0†
Selegiline	8	5	0	0	0	0	0	0
Antipsych	5	0	0	0	17	6	0	0
ChE Inhib	4	5	0	0	0	6	0	0
BZD	0	0	0	0	0	0	27†	0
LEU (range)	619 (0-3000)	250 (0-750)	407 (0-1050)	381 (0-1100)	0	453 (0-1100)	0	0
Clinical features								
MMSE (range)	27.7 (13-30)	25.9* (19-30)	25.8* (22-230	28.5 (27-30)	21.8* (17-28)	24.9* (0-30)	28.4 (26-30)	-
UPDRS II (range)	13.1 (2-30)	15 (6-21)	22* (10-32)	15 (0-30)	13.6 (8-24)	11.7 (0-26)	2* (0-8)	-
UPDRS III (range)	28.8 (7-60)	33.9 (14-60)	35 (31-42)	33 (3-55)	36.2 (24-60)	28.4 (4-74)	4* (0-13)	-
H&Y (range)	2.5 (1-5)	3.4* (2-4)	4* (2-5)	2.8 (1-4)	3 (2-4)	2.5 (1-5)	0.8 (0-2)	-

Table 11.1 Patient demographics* *vs. PD t-test $p<0.05$; † vs. PD χ^2 or Fisher's exact $p<0.05$* Medication use expressed in %; DA, dopamine agonist; Antichol, anticholinergic medication; Antipsych, antipsychotic medication; ChE Inhib, cholinesterase inhibitor; BZD, benzodiazepine medication; MMSE, mini-mental state examination; UPDRS, unified Parkinson's disease rating scale; H&Y, Hoehn and Yahr score; NR, not recorded

The screening question (QSVHI question A1) identified only 43 (38%) patients with clinically diagnosed PD who had VH, and none with PSP, MSA, VP or ET (table 11.2). Two (14%) control patients answered yes to this question, describing what transpired to represent visual auras associated with migraine. Eighty-five patients (75%) with PD, 3 patients (20%) with VP and three patients (14%) with PSP reported minor hallucinations and illusions (QSVHI question B), whereas none were reported in MSA. Amongst those with UP, nine patients (47%) experienced VH. Isolated auditory hallucinations (AH) occurred in 16% PD patients and none of the other movement disorders. VH were definitively related only to medications in 3% (two patients) of the PD hallucinators but medications were deemed responsible in 2 of 3 of PSP hallucinators. Delirium was associated with VH in 7% (5 patients) of PD hallucinators. In many

patients the time of onset of hallucinations could not be accurately recalled for example in the PD group only 68% of hallucinators could recall the time of onset of hallucinations.

QSVHI questions	PD	PSP	MSA	VP	DLB	UP	Disease control	Control
A General question	38	0*	0*	0	83	21	0*	14
B1 Presence	46	9*	0*	20	100*	22*	7	0
B2 Passage	52	5*	0*	0*	83	28*	20	7*
B3 Illusions	47	5*	0*	20	50	11*	20	0
B Total	72	9*	0*	20	100*	37*	20	7*
C1 Formed VH	38	0*	0*	20	67	11*	0*	0
C2 VH, associated AH	6	0	0	0	17	0	0	0
Total VH	75	14*	0*	20	100*	47	33	7*
D Auditory hallucinations	16	0*	0	0	17	11	7	0
E1 Associated delirium	5	0	0	0	17	0	0	0
E2 Medication related	2	9	0	0	0	0	20	0
Total VH - E = De-novo VH	68	5*	0*	20	83	47*	13	7*
Current VH	78	67	-	0	100	71	40	100
Past VH	22	33	-	100	0	29	60	0
VH Duration (range) *mths*	29 (1-144)	85 (6-240)	-	12 (12)	43 (6-130)	36 (1-120)	12 (1-28)	NR

Table 11.2 Frequency of "yes" response to QSVHI questions. * *vs. PD* χ^2 *or Fisher's exact* $p<0.05$. Results expressed as %; VH, visual hallucinations; AH, auditory hallucinations; NR, not recorded

De-novo VH, defined as VH not related to delirium or medications, were present in 78 (68%) PD patients but in only one (5%) patient with PSP. The PSP patient reported minor *presence* hallucinations that had been lifelong. Two patients with PD reported similar paranormal experiences going back to childhood. In disease controls the majority of VH were related to anticholinergic medications. Two (22%) disease control patients and one (7%) normal control reported *presence* hallucinations, limited to the months following the death of their spouse.

In PD hallucinators the MMSE was not significantly different to non-hallucinators (27.5 vs. 28.2, *t-test* $p=0.32$). However, PD hallucinators had significantly worse mean UPDRS II (14 vs. 10.7, *t-test* $p=0.02$), UPDRS III (30.6 vs. 24.7, *t-test* $p=0.04$) and Hoehn and Yahr (2.6 vs. 2.2, *t-test* $p=0.01$) scores. Amongst those with PD, disease duration was significantly longer in the hallucinators (11.3 vs. 6.6 years, *t-test* $p<0.001$).

There was no difference in frequency or dose of medication use between PD hallucinators and non-hallucinators.

The sensitivity and specificity of a positive response to individual QSVHI questions is summarized in table 11.3. The screening question (A) was as good as asking specifically for formed VH (question C1) in identifying patients with clinically diagnosed PD. The addition of minor hallucinations and illusions to the inventory (question B) doubled the sensitivity for PD, at the expense of specificity and PPV. However, the overall specificity of de-novo VH remained above 90%. When compared in patients where the diagnostic dilemma was between PD and PSP, the specificity of de-novo VH was 95% and the PPV approached 100%.

QSVHI question	PD vs. non-PD				PD vs. PSP			
	sens	spec	PPV	NPV	sens	spec	PPV	NPV
A Screening question	**36**	**100**	**100**	**38**	**36**	**100**	**100**	**23**
B1 Presence	45	89	91	38	45	91	96	24
B2 Passage	50	98	98	43	50	95	98	26
B3 Illusions	45	91	93	39	45	95	98	24
B Total	**72**	**89**	**94**	**55**	**72**	**86**	**97**	**37**
C1 Formed VH	36	93	93	36	36	100	100	23
C2 VH, associated AH	8	100	100	29	8	100	100	17
Total VH	**74**	**91**	**96**	**57**	**75**	**86**	**97**	**39**
Total de-novo VH	**62**	**91**	**95**	**48**	**62**	**95**	**99**	**32**

Table 11.3 QSVHI in PD and other bradykinetic rigid syndromes. Sens, sensitivity; spec, specificity; VH, visual hallucinations; AH, auditory hallucinations

Discussion

The QSVHI is a sensitive and simple method for establishing the presence of minor and major VH in Parkinsonism. It doubles the sensitivity of standard clinical screening questions and can be used in routine clinical practice. VH, as assessed by the QSVHI, are highly specific for the clinical diagnosis of PD. Hallucinators were found to have longer disease duration and more severe motor symptoms than non-hallucinators in PD but MMSE and medication use did not differ.

The PPV for PD has been determined as 98.6% by examination of pathological material. (Hughes *et al.*, 2002) In the present study the clinical diagnosis preceded the QSVHI implementation. De-novo VH assessed on the QSVHI were 91% specific for a clinical diagnosis of PD. Taken together this implies that patients who experienced VH according to the QSVHI have a 90% chance of having underlying Lewy body pathology.

Fenelon's minor hallucinations and illusions are not examined in detail in the VH components of the UPDRS (Fahn *et al.*, 1987), NPI (Cummings *et al.*, 1994) or other published questionnaires, including the Baylor PD Hallucination Questionnaire (Brandstaedter *et al.*, 2005) and the Parkinson Psychosis Questionnaire. (Fenelon *et al.*, 2000; Ondo *et al.*, 2005) Minor or "benign" hallucinations and illusions form part of the early spectrum of disorders of perception that eventually lead to formed VH in PD. (de Maindreville *et al.*, 2005; Goetz *et al.*, 2006) The QSVHI was designed to take into account the clinical observation that minor hallucinations and illusions occur frequently in PD but are not spontaneously reported (Fenelon *et al.*, 2000) and may be missed using only screening questions. Specific questioning for Fenelon's minor hallucinations (QSVHI questions B1, B2 and B3) substantially increased the number of VH reported compared to a typical screening that might be used in the UPDRS or NPI (QSVHI question A). This finding may prove particularly helpful in the UP group where QSVHI question A identified VH in 21%, but the complete QSVHI identified VH in 47% of UP patients. Given the specificity of VH for clinically diagnosed PD in this series, the majority of UP hallucinators are likely to have Lewy body pathology. For example, both patients in whom the differential diagnosis was between DLB and PSP reported VH on the QSVHI, favoring DLB as the underlying diagnosis. Of the seven UP patients with poor or no therapeutic levodopa response four reported VH on the QSVHI, favoring the diagnosis of PD rather than MSA or PSP. Two of the five patients where the diagnostic conundrum lay between PD and VP experienced hallucinations. The impact of the QSVHI on differential diagnosis is likely to be significant in the 4-5% of patients attending specialist clinics whose Parkinsonism remains unclassifiable. (Katzenschlager *et al.*, 2003)

VH are known to occur in up to a third of patients with AD (Scarmeas *et al.*, 2005) and secondary to anticholinergic medications, and in these clinical contexts the

inventory may be of limited use. In controls minor VH were reported by one person, suggesting that question B may detect paranormal phenomena and in patients with visual loss and Charles Bonnet syndrome VH are likely to be reported. It follows that in an unselected population the "false positive" rate could be high and specificity is likely to be somewhat lower than found here.

The QSVHI does not replace detailed clinical evaluation and assessment of the patient but it can be included easily in the routine assessment of patients being followed up in neurology clinics and will pick up far more patients experiencing VH than would be spontaneously reported at the consultation by the patient or the family.

One of the potential weaknesses of this study is the selection bias inherent in a movement disorders clinic from a tertiary referral centre. Given that the community prevalence of PD is estimated to be more than 20 times higher than PSP and MSA (Schrag *et al.*, 1999; Porter *et al.*, 2006), PD patients are probably under represented in this study. However, having more PD patients would have increased the PPV in this study. AD never presents with Parkinsonism but in any clinic with AD patients the PPV of QSVHI for PD would be lower than found in this study. The QSVHI was applied to a selected group of patients and is likely to be most useful where the diagnostic dilemma is between PD and PSP or MSA, a common diagnostic difficulty in specialist clinics. (Katzenschlager *et al.*, 2003) The lack of pathological confirmation also limits the conclusions that can be drawn from these findings, but the historical diagnostic accuracy in this clinic is known, so reasonable assumptions can be made. (Hughes *et al.*, 2001)

11.1.2. Olfaction in PSP

Background

Olfactory dysfunction is a common early finding in PD. A number of studies have found that olfaction is normal in PSP and have proposed that smell identification testing can differentiate between PD and PSP. (Doty *et al.*, 1993; Wenning *et al.*, 1995b) The underlying pathological substrate for hyposmia in PD is incompletely understood, but olfactory dysfunction can occur in other neurodegenerative disorders including PDC Guam (Doty *et al.*, 1991), Alzheimer's disease (Doty *et al.*, 1991), Huntington's disease and MSA. Previous studies using smell identification testing in PSP were performed prior to the publication of the NINDS-SPSP clinical diagnostic criteria and used data that was not adjusted for age or gender.

Aims

The aim of this study was to examine the diagnostic usefulness of smell identification testing using age and gender adjusted scores in a group of patients with PD and PSP diagnosed according to the UKPDSBB and NINDS-SPSP diagnostic criteria respectively. The diagnostic potential was also tested in a group of patients with unclassifiable Parkinsonism.

Methods

Data collection

Data was collected from patients attending the movement disorders clinic at the National Hospital for Neurology and Neurosurgery, London UK, where the University of Pennsylvania Smell Identification Test (UPSIT) is part of the routine clinic workup of new patients. Records from patients diagnosed with PD, according to the UKPDSBB criteria (Gibb and Lees, 1988), and clinically probable or possible PSP according to the NINDS-SPSP criteria (Litvan *et al.*, 1996a) were examined. A third group of patients with "unclassifiable" Parkinsonism (UP) was also included. These patients had either not satisfied any criteria for the diagnosis of PD, PSP, MSA, VP or CBD, or had features of several different diseases. Some of these patients have previously been reported.

(Katzenschlager *et al.*, 2003) Data including patient demographics, disease duration, and disease severity (UPDRS II and III and MMSE), smoking history and UPSIT scores were recorded.

Statistical analysis

Clinical characteristics and UPSIT scores amongst different disease groups were compared. Percentile estimates for age and gender using published data from North American controls were used and compared between the groups. Univariable analyses using χ^2 for categorical and two-tailed *t* test or the Mann Whitney U test, as appropriate, for continuous variables were applied.

Further analysis was performed in subgroups of PD and PSP with MMSE >21 and disease duration <8 years. This separation was done to replicate the population in whom UPSIT testing might be most useful, that is those with disease duration within the mean disease duration of PSP, and who have the cognitive ability to accurately perform the test.

A cut-off percentile ranking of UPSIT scores was determined for the diagnosis of PD and its sensitivity and specificity was calculated in a 2 x 2 table as follows. This was also calculated for PD vs. PSP.

	PD present	PD absent
UPSIT <12th percentile	a	b
UPSIT >11th percentile	c	d

Sensitivity = a/a+c
Specificity = d/b+d
PPV = a/a+b
NPV = d/c+d

Results

Clinical details were recorded from 64 patients with PD, 12 with NINDS-SPSP probable PSP and four with NINDS-SPSP possible PSP. Three of these patients were classified as PSP-P and the remaining 13 as RS, according to the clinical features in the first two years of disease. All PSP-P patients were initially diagnosed as PD, had a good response to dopaminergic medications, rest tremor and developed falls and supranuclear

gaze palsy more then five years after disease onset. One of these patients had previously been reported as unclassifiable Parkinsonism. (Katzenschlager *et al.*, 2003) Eight patients with UP were included in the study. In three of these patients the principal diagnostic dilemma was between PD and VP and in two it was between PSP, MSA and PD. Amongst the 3 other unclassifiable patients in one, where the differential diagnosis was between CBD and PD, a post mortem examination revealed both pathological processes and in another a +29 tau mutation has been identified.

There were no significant differences between mean ages, UPDRS or MMSE scores in PD compared to PSP, but mean disease duration was significantly shorter in PSP (table 11.4).

	PD	**PSP**	***p***	**PSP-P**	***p***
N	64	16		3	
Male %	64	56	***NS***	0	***0.026***
Age *yrs*	63.6 (37-81)	68.2 (62-80)	***NS***	69.9 (70-80)	***NS***
Disease duration *yrs*	10.4 (1.5-19)	5.2 (2-18)	***<0.001***	10.5 (6-18)	***NS***
UPDRS II	14 (2-27)	14 (6-21)	***NS***	17 (10-21)	***NS***
UPDRS III	30 (12-62)	29 (14-46)	***NS***	32 (18-39)	***NS***
H&Y	2.6 (1-4)	3.2 (2-4)	***0.03***	3.7 (3-4)	***0.035***
MMSE	28.1 (19-30)	27.1 (19-30)	***NS***	28.6 (27-30)	***NS***
UPSIT raw-score	17.6 (8-36)	24.7 (14-35)	***<0.001***	26 (22-27)	***0.017***
UPSIT percentile	9 (<5-23)	23 (<5-59)	***<0.001***	27 (13-54)	***<0.001***

Table 11.4 Patient demographics. *p*, p value for Student's t-test or χ^2; NS, not significant; H&Y, Hoehn and Yahr

Mean UPSIT raw-scores were significantly lower in PD compared to PSP; however there was substantial overlap (figure 11.1). When adjusted for gender and age, using published percentile data, there was less overlap (figure 11.2). The differences between means for PD and PSP-P were also significant (table 11.4, figure 11.2).

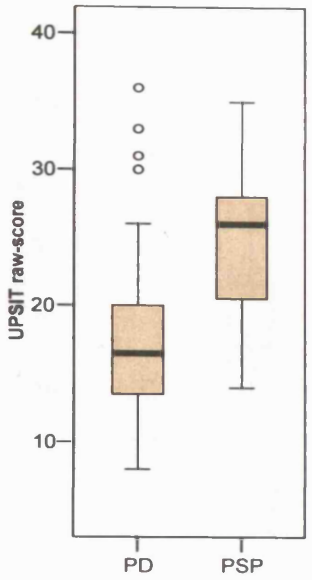


Figure 11.1
UPSIT raw-scores in PSP and PD.
Mean, interquartile range, range and outliers

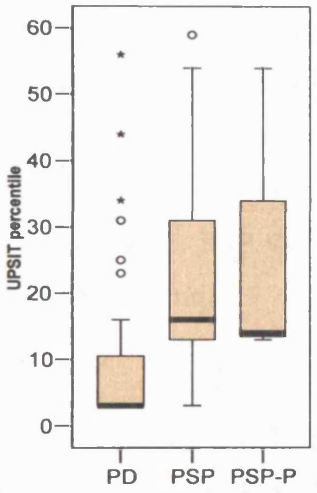


Figure 11.2
UPSIT age and gender percentile in PSP, PD and PSP-P.
Mean, interquartile range, range, and outliers.

. Eleven PD patients and 14 PSP patients with disease duration <8 years and MMSE >21 and were compared. Mean UPSIT raw-score of this PD sub-group was significantly less than the PSP sub-group (16.3 vs. 24.9, *Mann Whitney U test =0.001*). Mean percentile rankings were significantly different (5.7 vs. 21.0, *p=0.002*), but there was one patient whose scores overlapped with the PD range (figure 11.3). No PD patient in this subgroup had a UPSIT score ranked higher than the 11th percentile.

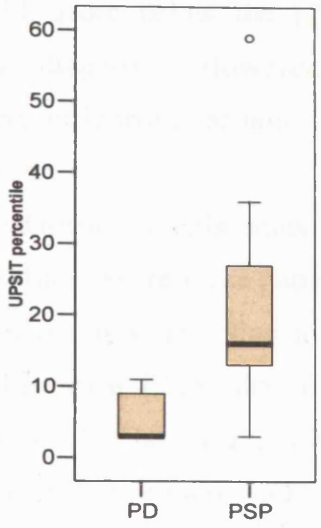


Figure 11.3
UPSIT age and gender percentile in PD and PSP (only patients with disease duration <8 years and MMSE >21).
Mean, interquartile range, range and outlier.

An UPSIT score below the 12th percentile for age was considered to be predictive of PD. In all patients with PD and PSP with MMSE >21, the specificity of an UPSIT score below the 12th percentile for PD was 88%, the sensitivity was 83%, the PPV was 96% and NPV was 54%. Amongst the patients classified UP, six (1: +29 tau mutation PD; 3: PD vs. VP; 1: PSP vs. MSA vs. PD) had UPSIT scores below the 12th percentile and three patients (1: PD vs. VP; 1: PSP vs. MSA vs. PD; 1: path confirmed CBD and PD) had scores above the 11th percentile.

Discussion

Patients with clinically diagnosed PSP performed better on smell identification testing than those with PD. In this group of selected patients, without a pathological diagnosis, the mean UPSIT scores were significantly different between PD and PSP, and this difference was greater when raw-scores were adjusted for age and gender. A cut-off UPSIT score set at the 11th percentile for age and gender had a high PPV for the clinical diagnosis of PD, but the specificity was only 88%. Most patients with a clinical diagnosis of PSP scored above this cut-off suggesting that testing with the UPSIT may be clinically useful in separating PSP form PD when the MMSE is greater than 21. Furthermore in three patients clinically categorised as PSP-P, mean UPSIT scores were significantly higher than PD. The specificity of this test means that in isolation it is unlikely to provide a highly accurate diagnosis but it may be helpful in certain clinical scenarios. For example this study predicts that in UP patients with an MMSE greater

than 21 and an UPSIT score below the 12th percentile, PSP is unlikely to be the underlying pathological diagnosis. However, the converse is not true and an UPSIT score above the 11th percentile would be non-contributory to the diagnosis, because of the range of scores seen in PD.

One of the weaknesses of this study is the selection bias associated with the tertiary referral centre clinic where these patients were tested. The lack of pathological confirmation is another obvious limitation to the conclusions that can be drawn from these findings, but the historical diagnostic accuracy in this clinic is known, so reasonable assumptions can be made. (Hughes *et al.*, 2001) A prospective study with pathological follow-up is needed to corroborate these findings.

11.1.3. The auditory startle response in the diagnosis PSP

Background

Electrophysiological tests including the acoustic startle response (ASR) and auditory blink reflex (ABR) have been proposed as a method of distinguishing PSP from other Parkinsonian conditions. (Litvan *et al.*, 1996a) The startle response is generated in the nucleus reticularis pontis caudalis which activates the reticulospinal tract inducing muscle responses in facial and spinal neurons. (Davis *et al.*, 1982) Neuronal loss in patients with PSP affects these structures and specifically involves cholinergic neurons of the lower pontine reticular formation, including those of the pedunculopontine tegmental nucleus and the nucleus pontis caudalis. (Zweig *et al.*, 1987; Juncos *et al.*, 1991; Malessa *et al.*, 1991) This distribution of pathology does not occur in other Parkinsonian disorders. The ABR, on the other hand, is thought to originate from different brainstem structures, and a number of studies have found that in PSP it can be retained even when the ASR is absent. (Kofler *et al.*, 2001; Gironell *et al.*, 2003) Animal studies of brainstem transections indicate that the pathway of ABR exists between the lower midbrain and the upper medulla, and the acoustic reflex pathway is postulated to include the superior olivary complex, the lateral lemniscus nuclei and the caudal colliculus in the midbrain. (Hori *et al.*, 1986)

Measurement of the ASR has been shown to differentiate NINDS-SPSP probable PSP from PD (Vidailhet *et al.*, 1992) and MSA. (Valldeoriola *et al.*, 1998) These studies suggested that ASR might be a useful diagnostic tool, particularly in differentiating the PSP from PD and other unclassifiable Parkinsonian syndromes. (Litvan *et al.*, 1996a) One prospective study assessed patients with MSA, CBD, VP as well as PSP. Patients were diagnosed using operational diagnostic criteria and sensitivity and specificity of ASR together with ABR and electro-oculography for the diagnosis of PSP were reported as 100% and 95% respectively. (Gironell *et al.*, 2003) The ASR was absent in all the PSP cases, in contrast to other studies where a range of responses were found.

These studies have been limited to clinically diagnosed patients without confirmatory pathological examination. Furthermore, patients without a definitive

diagnosis or with unclassifiable Parkinsonism have not been included, so the discriminatory ability of these tests is unknown in patients where the clinical diagnosis is uncertain. Finally, no attempt has been made to distinguish between the ASR and ABR in orbicularis oculi in different conditions, even though the contrasting reflex circuits would argue for a differential sensitivity to pathological processes.

Aims

The ASR and ABR have been studied in patients with PSP, including those with the PSP-P/PAGF phenotype, and unclassifiable Parkinsonism, to determine the diagnostic usefulness.

Patients and methods

Patients

Thirty-nine patients were recruited from the movement disorders clinic at the National Hospital for Neurology and Neurosurgery, London UK. The study was approved by the local research ethics committee. In all cases written informed consent was obtained prior to testing. Ten patients were diagnosed with PD according to the UKPDSBB diagnostic criteria (Gibb and Lees, 1988), one with DLB (McKeith *et al.*, 1996), 14 with NINDS-SPSP probable PSP (RS), five with NINDS-SPSP possible PSP (with a clinical phenotype consistent with PSP-P or PAGF) and nine with unclassified or undetermined Parkinsonism (UP). In one patient with RS the pathological diagnosis of PSP was confirmed.

Seven PD patients were recorded OFF medication and three were only recorded in the ON state. In two patients recordings were taken both off medications and 90 minutes following medications. Comparisons between on and off treatment responses were not statistically different in preliminary Mann Whitney U tests ($p > 0.05$), and all PD results were subsequently pooled together. In each patient demographic details, disease duration, pharmacological therapy, MMSE and UPDRS activities of daily living and motor scores were recorded. Patients with a MMSE score less than 21 were excluded. Twelve age matched control subjects, without neurological disease, were also tested. Two neurologists working at QSBB and blinded to the electrophysiological findings were

asked to rate, on a 10 point visual analogue scale, the likelihood of each patient with UP having PSP-tau pathology underlying their clinical disease. A mean grading of these results was used to classify the UP patients.

Methods

The patients were seated in a comfortable chair with arm and backrests, in a quiet room with an oil projector (Mathmos, London, UK) providing a relaxing visual stimulus to discourage sleep. A protocol modified from Brown (Brown *et al.*, 1991) and from Gironell (Gironell *et al.*, 2003) was used to obtain the ASR and ABR. A startle stimulus was delivered when the subject was relaxed with eyes open. Subjects received an auditory tone burst of 1 KHz, 150 ms duration and 110 dB from a custom made gated tone generator and delivered binaurally through earphones at pseudo-random intervals during one 15 minute epoch. Stimuli were separated by at least 60 seconds and by no more than 180 seconds, giving 5 responses in one epoch.

Electromyographic (EMG) activity was recorded using bipolar surface silver-silver chloride electrodes applied to the orbicularis oculi (OO) and sternomastoid (Scm) muscles bilaterally. For the OO, the two recording electrodes were placed 1 cm below and 1 cm medially from the external canthus. For the Scm, the recording electrodes were placed 2 cm apart over the midbelly of the muscle. EMG activity was sampled at 1000 Hz, amplified (X 5000) and filtered between 16 – 300 Hz using a D160 amplifier (Digitimer, Welwyn Garden City, UK) and 1401 analogue to digital (A-D) converter (Cambridge Electronic Design, Cambridge, UK). Raw EMG from each muscle was subject to DC removal (time constant 0.01s) before being rectified and averaged to the onset of the tone, with the 50 msec period before onset of the stimulus used as the baseline value in each of these sweeps.

Analysis of results

Analysis of results was performed blinded to clinical status. The presence of ASR in Scm and OO in the 5-trial average was examined. The ASR was considered to be absent if the amplitude of the EMG startle response was less than 5μV or 3 SD of the baseline mean (which ever was the greater). Onset latency (ASR) was measured as the

time interval between onset of acoustic stimulus and the onset (sustained increase of at least 10 contiguous points above 3 SD of the baseline mean) of the averaged EMG response in OO or Scm muscles. The duration of responses was measured from response onset until EMG activity returned to a consistent level below 3 SD of the baseline mean (at least 10 contiguous points). The size and peak amplitude of responses were also measured as percentage increases from the baseline mean activity. The OO was later found to be compound; consisting of both an early ABR and a later true startle reflex beginning at approximately 100 ms after auditory stimulation. Response duration and size was also analysed for these two components. Habituation was examined for each muscle by comparing the amplitude of responses to the first two versus the last two stimuli.

Statistical analysis

The frequency of the presence of ABR and ASR was compared between diagnostic groups (PD, PSP and controls). Mean values for ASR and ABR latency, duration and amplitude were calculated and pair wise comparisons between groups were performed. Data for UP were plotted against PSP and PD. The UP groups was dichotomised (unlikely PSP, <4 and possible or likely PSP, >3) and mean values of all parameters were compared. Univariable analyses using Fisher's exact test, for categorical and two-tailed *t* test or the Mann Whitney U test, as appropriate, for continuous variables were applied.

Results

Patient demographics

Table 11.5 shows the demographics of patients tested. There were significant differences in the MMSE, UPDRS and H&Y scores between the PSP and UP group. Overall the UP group had less severe disease. There was a significant difference in disease duration between RS (4.1 years) and PSP-P/PAGF (8.0 years, Student's t-test, *p=0.002*).

	Age (range)	Men (%)	Disease duration (range)	MMSE (range)	UPDRS		H&Y (range)
					II	III	
PD	63.9 (41-78)	90	8.4 (1-20)	27.7 (22-30)	12.3 (8-24)	35.3 (22-60)	2.4 (2-4)
PSP/PAGF	68.1 (52-81)	56	4.9 (1-11)	26.1 (19-30)	14.8 (8-20)	34.5 (14-55)	3.4* (2-4)
UP	63.8 (51-76)	70	6.8 (2-19)	29.2† (27-30)	5.6† (3-10)	18.8† (8-27)	2† (1-3)
Control	63.4 (48-74)	67	-	-	-	-	-

Table 11.5 Demographics in clinically diagnosed patients, *(PD vs. PSP/PAGF, t-test $p<0.05$), †(PSP vs. UP, t-test $p<0.05$)

Orbicularis oculi responses

Orbicularis oculi responses to a startling stimulus in controls typically consisted of two components. The first had a mean latency of 45 (SD 11) ms and was most likely the ABR due to its lack of habituation (figure 11.4). Grand averages revealed a second component occurring at approximately 100 ms, which was thought to reflect the true ASR (figure 11.4B).

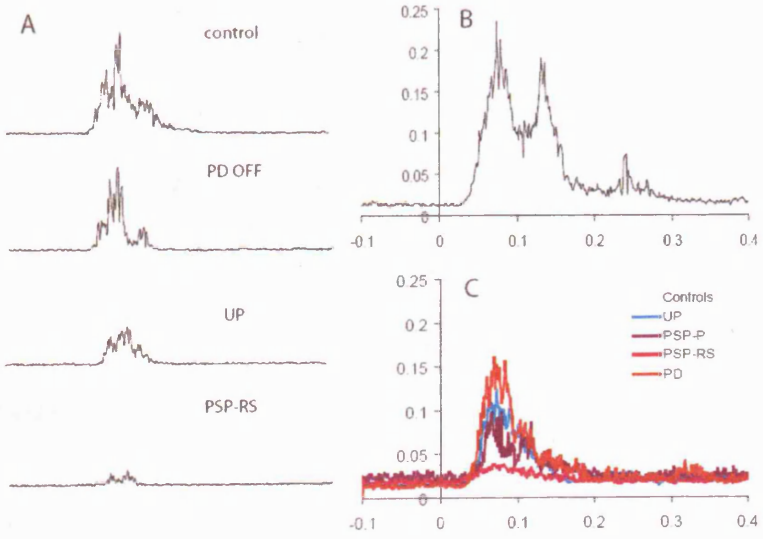


Figure 11.4 **A** Representative OO EMG recording from each group **B** Grand average of OO EMG from control group **C** Grand average OO EMG according to group

In controls both of these components were present in all but one person, in whom the OO ASR was absent (table 11.6). Both components were present in 9 (82%) PD patients. In PSP, both components were present in only four (21%) patients (2 (14%) RS and 2 (40%) PSP-P/PAGF, Fisher's exact, p>0.05) (table 11.6).

	PSP	PD	p	Controls	p	UP	RS	PSP-P/ PAGF	p
N	19	11		12		9	14	5	
OO response									
ABR present %	58	100	0.013	100	0.01	89	43	100	0.04
ASR present %	21	82	0.002	92	<0.001	67	14	40	NS
Scm response									
ASR present %	11	45	0.043	50	0.022	67	7	20	NS

Table 11.6 EMG response according to clinical diagnosis. Fisher's exact p value *vs.* PSP; NS, non significant difference

There were significant differences between mean ASR and ABR duration and amplitude in PSP and both controls and PD (figure 11.5; table 11.7). There were no significant differences between PD and controls.

	PSP	PD	*p*	Controls	*p*
ABR duration	0.023 (0.026)	0.101 (0.172)	0.006	0.052 (0.019)	0.006
ASR duration	0.018 (0.047)	0.067 (0.060)	0.005	0.092 (0.080)	< 0.001
ABR amplitude	365 (846)	985 (874)	< 0.001	1334 (923)	< 0.001
ASR amplitude	73 (155)	409 (305)	0.002	679 (857)	< 0.001

Table 11.7 Mean OO EMG parameters compared between groups. Duration *ms,* (SD); Amplitude % change from baseline; *p, Mann Whitney U vs.* PSP

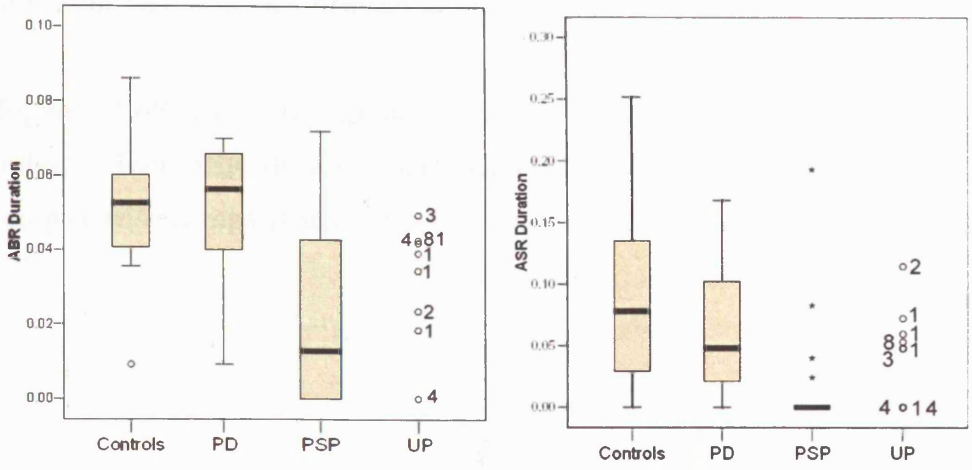


Figure 11.5A ABR and ASR duration (seconds) vs. clinical diagnosis. UP - grade of likelihood of PSP-tau pathology recorded next to case result (visual analogue scale 1-10)

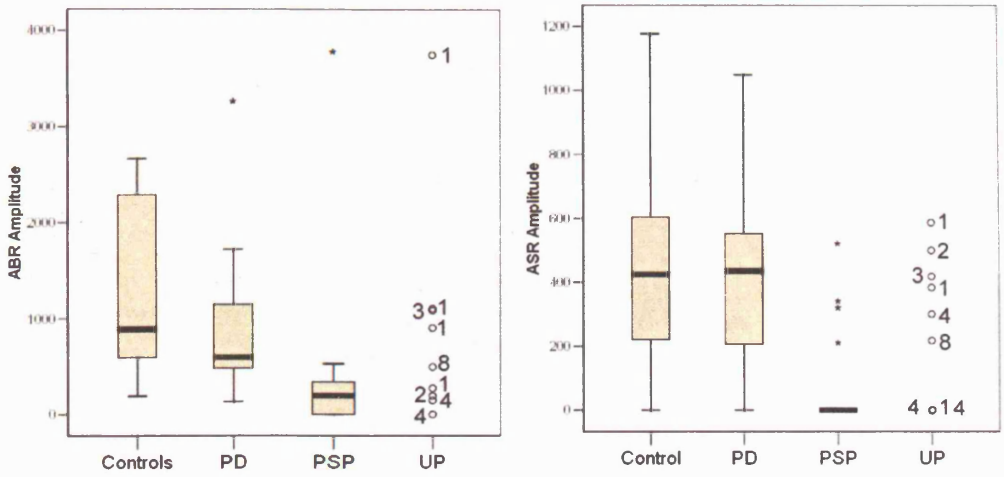


Figure 11.5B ABR and ASR amplitude (% change form baseline) vs. clinical diagnosis. UP - grade of likelihood of PSP-tau pathology recorded next to case result (visual analogue scale 1-10)

Sternocleidomastoid responses

Figure 11.6A shows typical Scm responses to startling stimuli in four subjects. The latency of the Scm response was longer than the OO response, occurring at mean 94 ± 11 ms after tone presentation in control subjects. Scm response showed considerable variability within groups, but tended to be smaller and of shorter duration in patients than

in controls. Scm response was present in only 50% of controls and fewer patients (table 11.6).

Figure 11.6B shows the grand averages for all groups normalized for baseline EMG activity. Scm ASR duration and amplitude values for PSP were significantly smaller on pair-wise comparisons with both controls and PD (table 11.8).

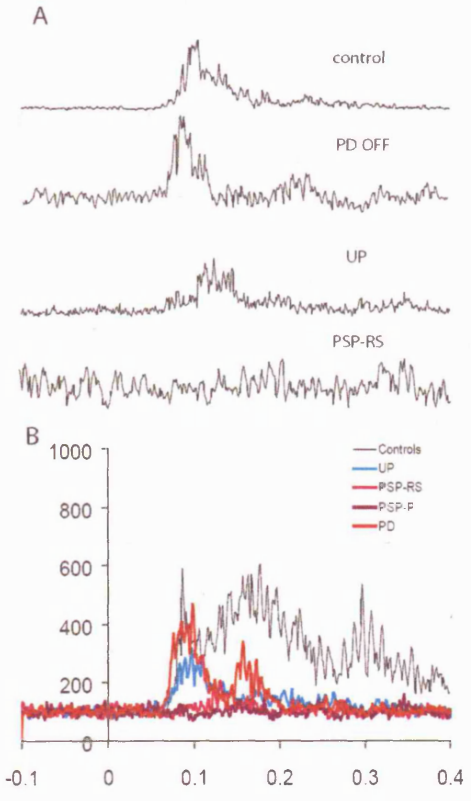


Figure 11.6 **A** Representative SCM ASR EMG **B** Grand average EMG according to group; %amplitude vs. time (sec)

	PSP	PD	*p*	Controls	*p*
Scm ASR duration	0.015 (0.051)	0.056 (0.066)	0.034	0.135 (0.155)	0.010
Scm ASR amplitude	40 (145)	199 (324)	0.030	329 (471)	0.015

Table 11.8 Mean Scm EMG parameters compared between groups. Duration, *ms* (SD); amplitude, % change from baseline; *p, Mann Whitney U vs.* PSP

Unclassifiable Parkinsonism

Nine patients were graded according to the likelihood of PSP-tau pathology underlying their clinic syndrome. Four patients were graded 1 or 2 (extremely unlikely to have PSP-tau pathology underlying), one grade three, two grade 4 and one graded 8 (likely to have PSP-tau pathology) and compared to PSP, PD and controls (figure 11.5). When dichotomised according likelihood of PSP there were no significant differences.

Discussion

Following a simple test protocol using a standardised acoustic startle the ABR and ASR were usually present in normal controls. The OO response was found to be complex, consisting of two peaks corresponding to the initial ABR and later OO ASR. In PSP both of these responses were usually absent, and, in particular, in RS both were present in only 14%. The OO ASR was almost universally absent in RS and PSP-P. In contrast the ABR was always present in PSP-P/PAGF, but was present in only 43% of RS. These findings imply that the extent of pathology in both PSP phenotypes extends to involve the ASR reflex circuits, but only in RS does it extend to structures in the ABR circuit. In PSP-P and PAGF the distribution of post mortem pathological tau is more limited than in RS, and the frequency of preserved startle and blink responses in PSP-P/PAGF compared to RS would support this (see chapter 8). In the PSP-P/PAGF group the mean duration of disease was twice that of RS, but the electrophysiological results imply the extent of pathological involvement was less in this group. Thus the nucleus reticularis pontis caudalis, where the ASR is generated, appears to be susceptible to damage in both RS and PSP-P/PAGF, but the midbrain structures involved in the ABR are significantly less susceptible in PSP-P/PAGF.

The SCM ASR was absent in some controls and PD, as well as PSP suggesting that neurodegeneration is not entirely responsible for these abnormalities. Qualitative analysis of OO and SCM responses showed significant differences between the means in patients with PSP, PD and controls. The amplitude and duration of ABR and ASR were consistently lowest in the PSP group, but the discriminatory ability of these findings is limited because of the degree of overlap between groups, and absent responses in controls. The diagnostic utility of the ABR and ASR can be estimated by examining the

range of results for PSP, PD and controls and comparing them to patients with UP. By definition clinical diagnosis is difficult in these patients and the value assigned for "likelihood" of PSP-tau pathology underlying the clinical syndrome remains the best guess. There was no consistent pattern of abnormality in individual patients and the results in UP patients graded 1 (extremely unlikely to have PSP-tau pathology) were similar to those graded greater than 3 (possible to likely).

The place of ASR and ABR testing remains uncertain in the diagnosis of PSP. The electrophysiological characteristics of the two PSP phenotypes support the contention that brainstem pathology is more limited in PSP-P and PAGF than RS. A longitudinal study may be able to address this further by tracking the change in ASR, and more importantly ABR. Pathological confirmation and regional pathological comparison between RS, PSP-P and PAGF, where ABR differs, may provide further insights into the dynamics of PSP-tau pathological progression. According to the current findings other clinical parameters are likely to be more helpful in differentiating PSP from PD and UP.

11.2. Retrospective studies of clinical features in pathologically diagnosed patients

11.2.1. Clinical differences between RS, PSP-P & other types of Parkinsonism

Aims

To determine what factors might be helpful in the clinical diagnosis of PSP-P using data from the QSBB.

Methods

Patients

A retrospective analysis was performed using the clinical files of 877 patients archived at the QSBB. Patients were recruited from around the United Kingdom and died between 1988 and 2003. Seventy one patients were excluded who had a pathological diagnosis other than PD, MSA, PSP, CBD, cerebrovascular disease (CVD), AD or PEP and 48 patients (5.5% of total) could not be included in the analysis because of insufficient clinical data. The remaining 758 patients included PD and DLB, n = 490; PSP, n = 127; MSA, n = 90; CVD, n = 25; AD, n = 9; CBD, n = 9 and PEP, n=8. Of the 25 patients with only CVD on histological examination four had essential tremor, with a positive family history, and two had other confounding factors (neuroleptic exposure and alcohol related dementia) and were removed from analysis of the VP group. PSP was further divided according to the number of clinical features associated with each clinical phenotype: 86 classified as RS (supranuclear gaze palsy, abnormality of saccadic eye movements, cognitive changes and falls in first two years), and 37 as PSP-P (in the first two years, asymmetric onset with a therapeutic response to levodopa and tremor).

Clinical data collection

A systematic chart review was performed on all the case notes using the same methodology as described in chapter 6.

Analysis

The proportion of cases with each clinical feature was compared separately for cases with PSP-P and non-PSP-P, as well as individually with PD, MSA and VP, using the χ^2-test for proportions for a two-by-two contingency table. Cases diagnosed with RS were not included. If an expected cell value was less than five, Fisher's exact test was used. For each clinical feature the sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) were calculated. A specificity of >0.85 or a PPV of >0.85 were considered good discriminators. Analyses were carried out using SPSS (version 12.0.1) software. To assess the influence of each feature on the diagnosis of PD, MSA and VP the sensitivities (1 – [PSPP specificity]), specificities (1 – [PSPP sensitivity]), PPV (1 – [PSPP NPV]) and NPV (1 – [PSPP PPV]) were calculated.

Results

The mean age at disease onset of all patients was 61.3 years (range, 9.1 to 87.5) and the mean duration of disease was 13.3 years (range, 1.2 to 72.8); 459 patients were men and 299 were women (table 11.9). There were significant differences between PSP-P and other diseases for age of onset (MSA earlier and VP later), duration of disease (PD longer, MSA and VP shorter) and age at death (MSA earlier, PD and VP later) (table 11.9). The diagnostic accuracy was significantly higher in PD and MSA than PSP-P

	All cases (n=758)	PSP-P (n=37)	Non-PSP-P (n=631)	PSP-P *vs.* non-PSP-P *p value*	PD (n=490)	PSP-P *vs.* PD *p value*	MSA (n=90)	PSP-P *vs.* MSA *p value*	VP (n=19)	PSP-P *vs.* VP *p value*
Male (%)	440 (61%)	19 (51%)	409 (64%)		309 (63%)		41 (46%)		11 (58%)	
Age at onset (yrs)	61.1 (9.1-87.5)	64 (44-85.3)	61.3 (9.1-87.5)	***0.035***	61.0 (28.6-84.2)	*0.100*	56.3 (33.5-79.0)	***<0.001***	71.0 (59.4-81.3)	***0.002***
Duration of disease (yrs)	13.3 (1.2-72.8)	12.7 (5.3-29)	13.3 (1.2-72.8)	*0.078*	15.0 (1.4-39.9)	***0.020***	8.1 (2.4-16.5)	***<0.001***	10.3 (2.6-18.2)	***0.016***
Age at death (yrs)	74.4 (39.4-95.8)	76.6 (50-92.6)	74.5 (39.4-95)	***0.002***	76.1 (49.8-93.4)	*0.730*	64.4 (39.4-86.0)	***<0.001***	81.4 (72.4-90.8)	***0.035***
Clinical diagnosis correct (%)	588 (78%)	14 (38%)	496 (79%)	***<0.001***	461 (95%)	***<0.001***	61 (68%)	***0.002***	2 (11%)	***0.015***

Table 11.9 Demographics of patients in QSBB archives, including PSP (PSP-P only) and non-PSP cases (PD, MSA and VP)

Tables 11.10, 11.11 and 11.12 show the different proportions of PSP-P and non-PSP-P cases with each of the different clinical features. When comparing PSP-P with PD, the frequency of nine early features, 10 late features as well as hallucinations and grade of response to levodopa differed significantly (table 11.10). Individually none of these factors had a high sensitivity and PPV for PSP-P. However, three of these clinical features, when calculated with respect to a diagnosis of PD, appeared to be reasonable discriminators of PD. Late drug induced dyskinesias (specificity 0.92, PPV 0.99), late autonomic dysfunction (0.94, 0.99) and visual hallucinations (0.94, 0.99) occur in more than a third of PD patients and less than 10% of PSP-P patients.

When comparing PSP-P with MSA, the frequency of five early features and nine late features differed significantly (table 11.11). Late non-specific eye symptoms and supranuclear gaze palsy were good discriminators of PSP-P. Three other clinical features, when calculated with respect to a diagnosis of MSA, appeared to be reasonable discriminators of MSA. Early autonomic dysfunction (specificity 1.0, PPV 1.0), late autonomic dysfunction (0.94, 0.80) and late cerebellar signs (0.97, 0.38) occurred in more than 50% of MSA patients and less than 10% of PSP-P patients.

When comparing PSP-P with VP, the frequency of two early features and six late features differed significantly (table 11.12). Pyramidal signs were more common throughout the disease in VP than PSP-P and postural instability was more frequent late in PSP-P but had a low specificity. Individually none of the other clinical factors had a high sensitivity and PPV.

Criteria	PSP-P %	PD %	*χ^2 p value	Sensitivity	Specificity	PPV	NPV
Early features							
Falls	3	5	NS	.03	.95	.04	.93
Bradykinesia	89	85	NS	.89	.15	.08	.95
Cognitive dysfunction	14	10	NS	.14	.90	.10	.93
Tremor	51	75	0.001	.51	.25	.05	.87
Axial rigidity	27	7	<0.001	.27	.93	.22	.94
Limb rigidity	66	80	0.048	.66	.20	.06	.89
Asymmetric onset	46	82	<0.001	.46	.18	.04	.82
Postural instability	19	8	0.036	.19	.92	.14	.94
Vertical SNGP	0	4	NS	.00	.96	.00	.00
Abnormal saccades	9	13	NS	.09	.88	.17	.78
Dysarthria/dysphonia	30	11	0.001	.30	.89	.18	.94
Dysphagia	11	2	0.001	.11	.98	.33	.93
Non-specific eye symptoms	16	4	0.001	.16	.96	.23	.94
Limb dystonia	3	4	NS	.03	.96	.05	.93
Pyramidal signs	8	2	0.033	.08	.98	.21	.93
Drug induced dyskinesias	0	<1	NS	.00	.99	.00	.00
Alien limb	0	<1	NS	.00	1.00	.00	.00
Cerebellar signs	0	<1	NS	.00	1.00	.00	1.00
Autonomic dysfunction	0	6	NS	.00	.94	.00	1.00
Cortical sensory loss	0	0	-	.00	.00	.00	.00
Late features							
Falls	97	69	<0.001	.97	.31	.10	.99
Bradykinesia	100	99	NS	1.00	.01	.07	1.00
Cognitive dysfunction	60	69	NS	.59	.31	.06	.91
Tremor	58	86	<0.001	.58	.14	.05	.81
Axial rigidity	71	29	<0.001	.71	.71	.16	.97
Limb rigidity	92	99	0.004	.92	.01	.07	.70
Postural instability	97	74	0.003	.97	.26	.10	.99
Vertical SNGP	68	13	<0.001	.68	.87	.50	.93
Abnormal saccades	83	44	0.014	.83	.56	.32	.93
Dysarthria/dysphonia	89	75	NS	.89	.25	.10	.96
Dysphagia	75	57	0.039	.75	.43	.11	.95
Non-specific eye symptoms	56	24	<0.001	.56	.76	.15	.96
Limb dystonia	25	24	NS	.25	.76	.07	.93
Pyramidal signs	14	8	NS	.14	.92	.12	.93
Drug induced dyskinesias	8	53	<0.001	.08	.47	.01	.86
Alien limb	0	0	-	.00	.00	.00	.00
Cerebellar signs	3	<1	NS	.03	.00	.33	.93
Autonomic dysfunction	6	38	<0.001	.06	.62	.01	.89
Cortical sensory loss	0	<1	NS	.00	.99	.00	1.00
Hallucinations	6	53	<0.001	.06	.48	.01	.87
Levodopa Response							
Grade 1	28	4	<0.001	0.28	0.96	0.37	0.94
Grade 2	44	12	<0.001	0.44	0.88	0.23	0.95
Grade 3	17	37	0.014	0.17	0.63	0.03	0.90
Grade 4	11	47	<0.001	0.11	0.53	0.02	0.88

Table 11.10 PSP-P vs. PD

Criteria	PSP-P %	MSA %	*χ^2 *p* value	Sensitivity	Specificity	PPV	NPV
Early features							
Falls	3	22	0.010	.03	.78	.05	.66
Bradykinesia	89	79	NS	.89	.21	.32	.82
Cognitive dysfunction	14	3	0.027	.14	.97	.63	.72
Tremor	51	42	NS	.51	.58	.34	.74
Axial rigidity	27	15	NS	.27	.85	.43	.74
Limb rigidity	66	68	NS	.66	.32	.29	.69
Asymmetric onset	46	59	NS	.46	.41	.24	.65
Postural instability	19	28	NS	.19	.72	.20	.72
Vertical SNGP	0	2	NS	.00	.98	.00	1.00
Abnormal saccades	9	24	NS	.09	.76	.14	.66
Dysarthria/dysphonia	30	24	NS	.30	.76	.35	.71
Dysphagia	11	7	NS	.11	.93	.40	.70
Non-specific eye symptoms	16	1	0.001	.16	.99	.86	.74
Limb dystonia	3	6	NS	.03	.94	.17	.69
Pyramidal signs	8	16	NS	.08	.84	.18	.68
Drug induced dyskinesias	0	2	NS	.00	.98	.00	1.00
Alien limb	0	0	-	.00	.00	.00	.00
Cerebellar signs	0	13	0.03	.00	.88	.00	1.00
Autonomic dysfunction	0	54	<0.001	.00	.46	.00	1.00
Cortical sensory loss	0	0	-	.00	.00	.00	.00
Late features							
Falls	97	78	0.012	.97	.22	.34	.95
Bradykinesia	100	96	NS	1.00	.04	.30	1.00
Cognitive dysfunction	60	26	0.006	.59	.74	.49	.81
Tremor	58	61	NS	.58	.39	.28	.69
Axial rigidity	71	48	0.04	.71	.53	.37	.82
Limb rigidity	92	93	NS	.92	.07	.30	.67
Postural instability	97	94	NS	.97	.06	.31	.83
Vertical SNGP	68	10	<0.001	.68	.90	.72	.89
Abnormal saccades	83	72	NS	.83	.28	.30	.82
Dysarthria/dysphonia	89	95	NS	.89	.05	.29	.50
Dysphagia	75	90	0.027	.75	.10	.27	.47
Non-specific eye symptoms	56	10	<0.001	.56	.90	.69	.83
Limb dystonia	25	35	NS	.25	.65	.23	.68
Pyramidal signs	14	57	<0.001	.14	.43	.10	.52
Drug induced dyskinesias	8	23	NS	.08	.77	.13	.67
Alien limb	0	1	NS	.00	.99	.00	1.00
Cerebellar signs	3	35	<0.001	.03	.65	.03	.62
Autonomic dysfunction	6	90	<0.001	.06	.10	.03	.20
Cortical sensory loss	0	1	NS	.00	.99	.00	1.00
Hallucinations	6	9	NS	.06	.91	.20	.71
Levodopa Response							
Grade 1	28	40	NS	.28	.59	.25	.63
Grade 2	44	30	NS	.47	.70	.44	.73
Grade 3	17	26	NS	.17	.74	.24	.64
Grade 4	11	4	NS	.11	.96	.57	.70

Table 11.11 PSP-P vs. MSA

Criteria	PSP-P %	VP %	*χ^2 *p* value	Sensitivity	Specificity	PPV	NPV
Early features							
Falls	3	21	0.040	.03	.79	.20	.29
Bradykinesia	89	79	NS	.89	.21	.69	.50
Cognitive dysfunction	14	21	NS	.14	.79	.56	.32
Tremor	51	53	NS	.51	.47	.66	.33
Axial rigidity	27	17	NS	.27	.83	.75	.38
Limb rigidity	66	94	NS	.66	.06	.58	.08
Asymmetric onset	46	58	NS	.46	.42	.59	.30
Postural instability	19	39	NS	.19	.61	.46	.31
Vertical SNGP	0	0	-	.00	.00	.00	.00
Abnormal saccades	9	0	NS	.09	.00	1.00	.00
Dysarthria/dysphonia	30	11	NS	.30	.89	.85	.40
Dysphagia	11	5	NS	.11	.95	.80	.35
Non-specific eye symptoms	16	0	0.045	.16	.00	1.00	.00
Limb dystonia	3	0	NS	.03	.00	1.00	.00
Pyramidal signs	8	33	0.044	.08	.67	.33	.27
Drug induced dyskinesias	0	0	-	.00	.00	.00	.00
Alien limb	0	0	-	.00	.00	.00	.00
Cerebellar signs	0	0	-	.00	.00	.00	.00
Autonomic dysfunction	0	0	-	.00	.00	.00	.00
Cortical sensory loss	0	0	-	.00	.00	.00	.00
Late features							
Falls	97	67	0.003	.97	.33	.74	.86
Bradykinesia	100	94	NS	1.00	.06	.69	1.00
Cognitive dysfunction	60	53	NS	.59	.47	.66	.41
Tremor	58	58	NS	.58	.42	.66	.35
Axial rigidity	71	33	0.02	.71	.67	.79	.57
Limb rigidity	92	100	NS	.92	.00	.65	.00
Postural instability	97	76	0.037	.97	.24	.72	.80
Vertical SNGP	68	20	NS	.68	.80	.93	.40
Abnormal saccades	83	100	NS	.83	.00	.91	.00
Dysarthria/dysphonia	89	53	0.014	.89	.47	.80	.64
Dysphagia	75	47	NS	.75	.53	.79	.47
Non-specific eye symptoms	56	5	0.004	.56	.95	.95	.53
Limb dystonia	25	16	NS	.25	.84	.75	.37
Pyramidal signs	14	47	0.020	.14	.53	.36	.24
Drug induced dyskinesias	8	16	NS	.08	.84	.50	.33
Alien limb	0	0	-	.00	.00	.00	.00
Cerebellar signs	3	0	NS	.03	.00	1.00	.00
Autonomic dysfunction	6	21	0.14	.06	.79	.33	.31
Cortical sensory loss	0	0	-	.00	.00	.00	.00
Hallucinations	6	5	NS	.06	.95	.67	.35
Levodopa Response							
Grade 1	28	28	NS	.28	.72	.67	.33
Grade 2	44	22	NS	.47	.78	.81	.42
Grade 3	17	33	NS	.17	.67	.50	.29
Grade 4	11	17	NS	.11	.83	.57	.32

Table 11.12 PSP-P vs. VP

Conclusions

Clinically PSP-P is often difficult to distinguish from PD and DLB, MSA and VP. PSP-P shares many clinical features with PD and DLB, but visual hallucinations, drug induced dyskinesias and autonomic dysfunction are very uncommon in PSP-P and are helpful exclusion criteria.

Cardiovascular autonomic failure does not occur in PSP-P and this probably accounts for the rare false positive diagnoses of MSA in this series. There were no particularly strong clinical discriminators between VP and PSP-P, although this study did not include imaging findings which are an essential part of the diagnostic criteria for VP. (Zijlmans *et al.*, 2004a)

11.2.2. Falls in PSP

Background

The diagnoses of PSP and PD are contingent on the presence of postural instability and falls. (Hughes *et al.*, 1992a; Litvan *et al.*, 1996c) In patients with disturbances of gait due to parkinsonism, fractures are more common than in those with gait disturbances due to other neurological conditions such as peripheral neuropathies. (Syrjala *et al.*, 2003) Despite the greater frequency of falls in PSP, falls and their proclivity to cause bone fractures has not been systematically analysed. (Wenning *et al.*, 1999; Wielinski *et al.*, 2005) Falls occur frequently in Parkinsonian conditions and are associated with a poorer quality of life. (Schrag *et al.*, 2001; Bloem *et al.*, 2004) The risk of bone fracture increases with a history of falls, impairment of mobility, low body mass index and low bone mineral density. (Greenspan *et al.*, 1998; Sato *et al.*, 2001)

Falling within the first year of disease is required by the NINDS-SPSP criteria for the diagnosis of probable PSP, although in the QSBB series less than two-thirds of patients with pathologically diagnosed PSP had fallen in the first year of disease. (Osaki *et al.*, 2004) Falls occur frequently in PSP-P, but usually later in the disease than RS. The stringent NINDS-SPSP diagnostic criteria use falls within the first 12 months of disease because of its high specificity for RS. (Litvan *et al.*, 1996a)

A number of factors contribute to falls in bradykinetic rigid syndromes and may help to distinguish them from PSP-P. Gait unsteadiness and cognitive impairment contribute to falls in CBD and DLB. In MSA, on the other hand, orthostatic hypotension and cerebellar or Parkinsonian gait disorder are important precipitants. (Wenning *et al.*, 1995a)

Aims

The aims of this study were to quantify the temporal evolution of falls in RS, PSP-P, PD, MSA, CBD, DLB, VP and AD and to analyse the relationship between time from disease onset to first fall (latency) and pathological diagnosis, specifically to

determine if latency is helpful in distinguishing PSP-P from PD. The relationship between pathological diagnosis and fractures was also investigated.

Patients and methods

Patients

A retrospective analysis was performed using the clinical files of 774 patients with a lifetime diagnosis of a bradykinetic rigid syndrome or Parkinsonism with a pathologically confirmed diagnosis (PD, n = 474; PSP, n = 127; MSA, n = 91; DLB, n = 46; VP, n = 19; AD, n = 9; CBD, n = 8) archived at the QSBB. A few cases (2% of total) could not be included in the analysis because of insufficient clinical data (10 PD, 3 PSP, 1 MSA, 1 AD, 1 VP). The PSP group were further divided according to the number of clinical features reported associated with each clinical phenotype, not including falls in this designation: RS (supranuclear gaze palsy, abnormality of saccadic eye movements, cognitive changes in first two years), PSP-P (tremor and non-axial dystonia in the first two years, asymmetric onset and response to levodopa).

Data collection

A systematic chart review was performed on all the case notes using the same methodology as described in chapter 6.

Statistical analysis

The report and timing of any falls from the time of disease onset until death or fractures was the primary outcome measure. Descriptive statistics were used to analyse the latency to first fall and frequency of different skeletal bones fractured in each diagnostic group. Clinical characteristics amongst different diseases were compared. The proportion of PD and PSP-P cases within latencies either side of a nominated cut-off according to findings on the descriptive statistics were compared using the chi-square test for proportions for a two-by-two contingency table. The sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) were calculated. The association between bradykinesia, rigidity, tremor, cognitive dysfunction, speech disturbance, dysphagia, dystonia, dyskinesias, autonomic dysfunction, gender, symmetry of clinical

features, visual hallucinations and age at disease onset with falls and fractures in all diseases was investigated. Univariable analyses using χ^2 for categorical and two-tailed t-test or the Mann Whitney U test, as appropriate, for continuous variables were applied. In PSP univariable analysis was performed on falls in the first two years (i.e. early falls, rather than any falls) because of the high frequency of falls. In PSP, RS and PSP-P the effect of falls on prognosis was assessed by calculating the mean disease duration in patients who fell in the first two years of disease and comparing it to those who fell after two years. Kaplan-Meier plots were used to graphically assess survival. Cox multiple stepwise regression analysis was performed in all diseases for latency to first fall, using clinical factors present around disease onset (within the first two years) and gender as categorical covariates and age at onset as a continuous variable.

Results

Amongst the 782 cases the incidence of falls from disease onset to death was 77.5%. It was highest in CBD (100%) and PSP (97.5%) and lowest in MSA (77.6%) and PD (73.3%). (Table 11.13) Falls occurred earlier in those with PSP (median 17 months; range 0-244 months) than all other diseases (figure 11.7).

	PD	PSP	MSA	DLB	CBD	VP	AD
Number	474	127	91	46	8	27	9
Mean age at onset, yrs (range)	60.4 (28.6-84.2)	65.7 (40.1-87.5)	56.4 (33.5-79)	66.7 (43.0-82.6)	66.2 (55.6-76.1)	69.6 (39.5-81.3)	69.4 (59.6-84.8)
Mean age at death, yrs (range)	76.4 (49.8-93.4)	73.7 (45.4-95.8)	64.4 (39.4-86.0)	74.9 (54.9-88.3)	73.0 (65.8-81.6)	82.5 (72.4-93.6)	80.2 (71.0-94.6)
Women (%)	39.9	37.3	53.8	20	20	48	44.4
Fallers (%)	73.3	98.3	77.6	58.1	100	73.9	85.7
Median time to first fall, months (range)	108 (0-384)	16.8 (0-244)	42 (0-165)	54 (0-158)	30 (0-90)	40.8 (0-304)	66.6 (36-228)
Fractures (%)	16.9	28.6	11	4.3	0	11.1	22.2

Table 11.13 Characteristics of patients according to pathological diagnosis

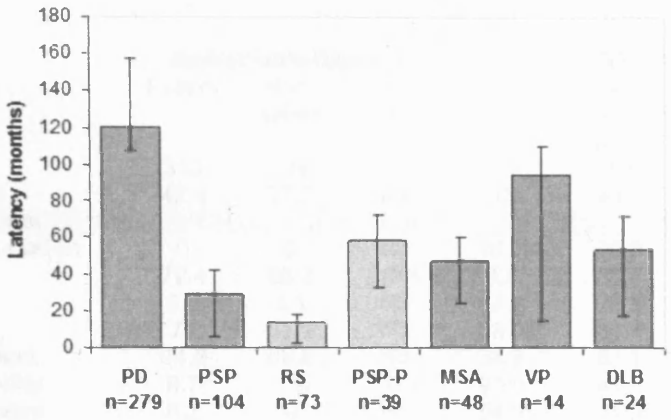


Figure 11.7 Latency to first fall according to disease, showing interquartile range

Median time to first fall was significantly shorter in RS (12; range 0-95) compared to both PSP-P (47; 0-228, $p<0.001$), PD (108; 0-384, $p<0.001$) and MSA (42; 0-165). A cut-off of 70 months was used to test the usefulness of falls in separating PSP-P from PD. In PSP-P 49% of patients fell within 70 months and in PD 19% fell within that time. For the diagnosis of PSP-P the sensitivity of this clinical feature was 0.48, the specificity was 0.81, the PPV was 0.18 and the NPV was 0.96.

In PSP univariable analysis showed an association between early falls (within the first two years of disease) and symmetrical disease onset and early cognitive dysfunction, axial rigidity, postural instability, eye movement abnormalities and speech disturbance (occurring within 2 years of disease onset). (Table 11.14) In common with PD, in those with early falls, tremor was less frequent. In PD there was also an association between the occurrence of falls and the late clinical features of cognitive dysfunction, speech disturbance, dysphagia, autonomic dysfunction and hallucinations and a negative association between the falls and early and late tremor. In MSA falls at any time were associated with the late clinical features of limb rigidity, speech disturbance, dysphagia and pyramidal tract signs. There were no significant associations between falls and clinical features in VP, DLB, CBD and AD; this may be due to the small sample sizes for these diseases.

	Parkinson's Disease				PSP			MSA	
	Fallers	Non-fallers	*p-value*	Early Fallers	Early non-fallers	*p-value*	Fallers	Non-fallers	*p-value*
Number	313	114		75	49		66	19	
Gender (% female)	43.1	27.2	0.002	36	40.8	NS	59	36.8	NS
Early clinical features									
Cognitive dysfunction	0	0	NS	70.7	28.6	<0.001	4.8	0	NS
Tremor	72.4	86.2	0.004	6.8	35.4	<0.001	42.2	42.1	NS
Axial rigidity	5.6	8.1	NS	54.7	32.5	0.024	16.7	5.9	NS
Limb rigidity	75	81.9	NS	63.9	61.4	NS	61.1	68.9	NS
Asymmetric onset	84.8	88.2	NS	34.8	83.1	<0.001	60.3	50	NS
Postural instability	8.7	1.9	0.017	91.9	41.9	<0.001	28.6	21.1	NS
Vertical gaze palsy	6.2	0	NS	54.9	11.5	<0.001			
Abnormal saccades	12	0	NS	83.3	45.0	0.002			
Speech disturbance	9.6	6.5	NS	66.7	41.7	0.006	19.3	38.9	NS
Dysphagia	0.7	2.9	NS	26.7	12.5	NS	4.9	17.6	NS
Pyramidal signs	3.3	0	NS	12.3	15.2	NS	17.4	16.7	NS
Dyskinesias	0.7	0.9	NS	0	0	NS	0	1.6	NS
Autonomic dysfunction	5.7	3.6	NS	1.4	2.0	NS	55.6	58.9	NS
Late clinical features									
Cognitive dysfunction	71.7	49.5	<0.001				31.6	12.5	NS
Tremor	85.7	93.6	0.018				60.3	68.4	NS
Axial Rigidity	30.1	22.3	NS				47.5	41.2	NS
Limb rigidity	98	98.2	NS				96.7	77.8	0.008
Postural instability	45.6	83.8	<0.001				94.7	88.2	NS
Speech disturbance	78.8	59	<0.001				98.4	82.4	0.007
Dysphagia	59.7	46.9	0.033				94.9	68.8	0.003
Dyskinesias	58.1	53.3	NS				22.2	22.2	NS
Autonomic dysfunction	46.9	21.5	<0.001				88.9	100	NS
Pyramidal signs	5.4	9.4	NS				66.7	33.3	0.018
Hallucinations	53.3	37.5	0.004				10.6	5.9	NS

Table 11.14 Clinical features in fallers and non-fallers according to pathological diagnosis. Percentage, χ^2

Multivariate analyses were performed using the Cox multiple stepwise regression model on early clinical features, age and gender. This identified several clinical factors which independently influence time to first fall (table 11.15).

PD	HR (95% CI)	p	PSP	HR (95% CI)	p	MSA	HR (95% CI)	p
Age of onset	1.07 (1.05-1.08)	<0.001	Age of onset	1.05 (1.01-1.09)	0.007	Age of onset	1.04 (1.02-1.08)	0.003
Symmetrical onset	1.86 (1.25-2.77)	0.002	Early postural instability	3.4 (1.7-6.8)	0.001	Early postural instability	2.08 (1.07-3.94)	0.03
Early autonomic dysfunction	2.56 (1.32-4.98)	0.005						
Female gender	1.39 (1.05-1.83)	0.021						

Table 11.15 Factors affecting time to first fall in PD, PSP and MSA: Independent predictors from Cox multiple regression analysis on early clinical features, age and sex. HR, hazard ratio; CI, confidence interval

In PD female gender, older age, symmetrical onset and autonomic dysfunction were all independent predictors of earlier falls. Age of onset was also an important independent factor predicting time to first fall in PSP and MSA, as was postural instability.

Almost all patients with PSP had falls at some point in their clinical history; patients with falls in the first two years had a mean life expectancy 3.3 years lower than other PSP patients (table 11.16). This difference was greater in the PSP-P group, and there was no difference in the RS group. This is illustrated graphically by the Kaplan-Meier plot (figure 11.8).

		Falls <2 y	Falls >2 y	p value
PSP	Duration, y	6.4	9.7	<0.001
	Onset, y	66.7	64.7	NS
RS	Duration, y	6.2	6.8	NS
	Onset, y	66.1	64.6	NS
PSP-P	Duration, y	7.1	11.0	0.005
	Onset, y	69.1	64.7	NS

Table 11.16 Disease duration (time from onset until death) and age at onset in early (<2 years) and late (>2 years) fallers in PSP, RS and PSP-P (Mann-Whitney U Test)

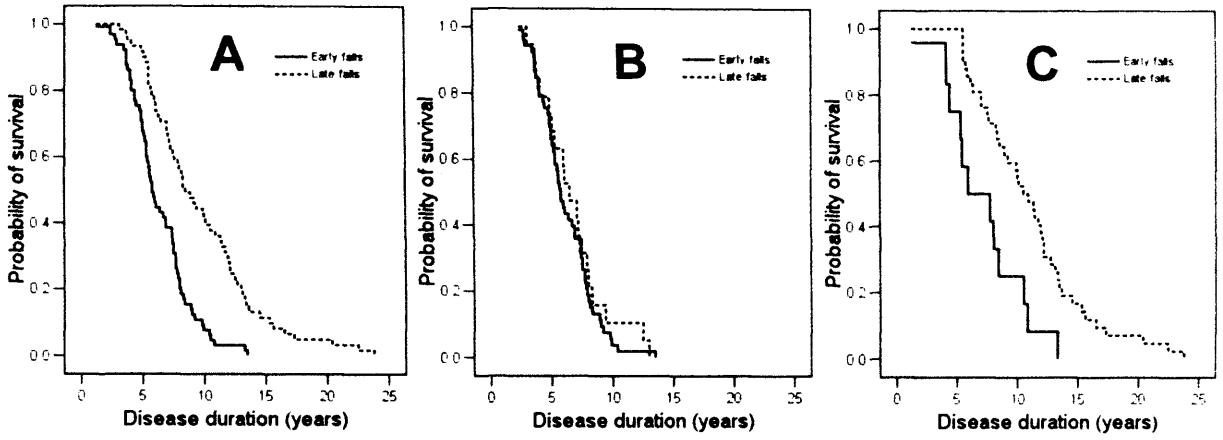


Figure 11.8 Kaplan-Meier plot illustrates the effect of early or late falls upon survival in 124 cases of (a) PSP in total, and separated into subgroups (b) RS (72 cases) and (c) PSP-P (54 cases)

There were 166 fractures recorded in 134 (17.1%) patients. Thirty six (28.6%) patients with PSP sustained a total of 52 fractures, 80 patients (16.9%) with PD sustained 96 fractures, and 10 (11%) patients with MSA sustained a fracture. Hip fractures comprised 46.9% of all fractures in PD and one third of fractures occurred in the upper limb. In PSP the proportion of hip fractures was less than in PD (30.7% vs. 46.9%, p=0.053 t-test), and the proportion of "other upper limb fractures" (including the clavicle, scapula and bones in the hand) was higher than in PD (p=0.109, t-test) (table 11.17). Comparing all patients (with and without fractures), multiple fractures were significantly more common in PSP than in both PD (6.4% vs. 1.9%, p=0.007 t-test) and MSA (6.4% vs. 0%, p=0.014 t-test). Skull fractures (3.2% vs. 0.8%, p=0.43 t-test) and truncal fractures (5.6% vs. 1.9%, p=0.24 t-test) were also more common in PSP than in PD. There were no substantial or significant differences in fracture site between RS and PSP-P.

	PD	PSP	RS	PSP-P	MSA	VP	DLB	AD
Forearm	17 (17.7)	7 (13.5)	5 (16.7)	2 (9.1)	3 (30)	1 (33)		
Humerus	10 (10.4)	5 (9.6)	2 (6.6)	3 (13.7)	1 (10)	1 (33)		
Other upper limb	6 (6.3)	8 (15.4)	5 (16.7)	3 (13.7)	0	1 (33)		1 (50)
Hip	45 (46.9)	16 (30.7)	10 (30.0)	7 (31.8)	4 (40)		2 (100)	
Distal leg	5 (5.2)	4 (7.7)	3 (10)	1 (4.5)	0			1 (50)
Pelvis	1 (1.0)	1 (1.9)	0	1 (4.5)	0			
Trunk	8 (8.3)	7 (13.5)	3 (10)	4 (18.2)	1 (10)			
Skull	4 (4.2)	4 (7.7)	3 (10)	1 (4.5)	1 (10)			
Multiple	9	8	7	5	0			
Total fractures	96 (100)	52 (100)	30 (100)	22 (100)	10 (100)	3 (100)	2 (100)	2 (100)

Table 11.17 Distribution of fracture location in bradykinetic rigid syndromes: Number (percentage of all fractures)

In PSP early bradykinesia (28.6% with fractures vs. 8% with fractures, χ^2 p=0.027), early limb rigidity (47% vs. 68.7%, χ^2 p=0.028) and early pyramidal signs (2.5% vs. 18.8%, χ^2, p=0.02) occurred less frequently in those with fractures. Gender distribution was not significantly different between fracture and non-fracture groups in PSP (34% women and 44% women respectively, χ^2 p=0.275). In contrast, women were over represented in PD fracture groups (28.6% vs. 11.3%, χ^2 p<0.001) and amongst the 10 MSA patients with fractures (90% vs. 49.4%, χ^2 p=0.015). The clinical features that were significantly associated with fractures in PD were early falls (9.3% with fractures vs. 3.2% no fractures, χ^2 p=0.024), late falls (100% vs. 66.7%, χ^2 p<0.001) and late postural instability (88.4% vs. 71%, χ^2 p=0.003). VP patients with fractures did not have bradykinesia (0% vs. 83% VP without fractures, χ^2 p=0.017) or limb rigidity (0% vs. 100% VP without fractures, χ^2 p=0.02).

Discussion

Using the "gold standard" of pathological diagnosis and retrospective case notes review methodology, several important factors related to falling have been identified which help to distinguish the most common neurodegenerative, bradykinetic rigid syndromes. Falls occurred within the first 3 years of disease onset in a majority of patients with PSP, within 4 years in MSA, 5 for those with DLB and a median of 9 years for those with PD.

There was a significant difference between the time to first fall in RS and PSP-P, when these clinical phenotypes were separated using the clinical features identified by factor analysis in chapter 6.1, but excluding falls. There was substantial overlap between the mean latency to first fall in PSP-P and MSA and VP, but there was a significant difference when compared to PD (see figure 11.7) Despite this, the sensitivity and PPV of falls before 70 months for PSP-P was low and in isolation unhelpful in separating these patients in the clinic.

Overall the incidence of falls and fractures was high (77.6% and 17.1% respectively). 73% of patients with PD had falls and 16.9% sustained fractures. A previous prospective survey of PD and elderly patients predicted a higher incidence of fractures caused from falls in PD. (Genever *et al.*, 2005) Studies have estimated an incidence of falls as high as 69% in PD and 32% in elderly patients. (Tromp *et al.*, 1998; Wood *et al.*, 2002) However, falls and fractures are likely to have been underestimated in the present study because it relied entirely on the accuracy and completeness of clinical notes. Patients with other bradykinetic rigid syndromes, including PSP, CBD and MSA, fall more frequently and earlier in the disease than in PD, and it has been suggested that these patients have a higher incidence of fractures. (Wenning *et al.*, 1999) More than three-quarters of patients with PSP (97%), MSA (77%) and CBD (100%) fell during their disease, though only 53% of patients with VP and 58% of patients with DLB fell. Compared to PD, the incidence of fractures was higher in PSP, but lower in MSA, CBD, VP, DLB and AD, partially reflecting the differences in disease duration and emphasising one of the strongest clinical characteristics of PSP-tau pathology.

Falls in PSP, RS and PSP-P

In PSP early falls were associated with symmetrical disease onset, early cognitive dysfunction, axial rigidity, dysphagia and eye movement abnormalities. These clinical factors reflect the importance of axial symptoms in balance and gait disturbance. Prognosis in PSP has previously been related to early falls, speech and swallowing problems and time to PEG tube insertion. (Nath *et al.*, 2003) There was a significant difference in disease duration between early fallers and late fallers in PSP and PSP-P. This difference was not found in RS. The Kaplan-Meier curves suggest that early falls in

PSP predict mortality, particularly in the PSP-P subgroup. In the general population the risk of falling increases with age and so too in PSP the age of onset had the greatest effect on the latency to first fall in the multiple regression analysis. Postural instability was also a significant factor, although given the retrospective nature of the study; it is possible that the recording of this clinical feature was more likely in those who had fallen than those who had not. Patients were selected on the basis that they died during a specified period of time. If the method or accuracy of diagnosis is changing over time, then this too may cause some bias in the type of patients included in this study. Furthermore, people diagnosed more recently would need to die sooner after diagnosis to be included.

Falls in PD

This study has confirmed previous reports of a high incidence of disturbances of higher cortical functions in PD fallers (Wood *et al.*, 2002; Marchese *et al.*, 2003), as indicated by the clinical parameters identified here, including: cognitive dysfunction and hallucinations, and axial symptoms, such as speech disturbance and dysphagia. Autonomic dysfunction and absence of tremor in PD were also found to be more frequent in those who fell. The lower incidence of tremor in PD fallers has previously been reported. (Koller *et al.*, 1989; Wood *et al.*, 2002)

Age of onset, gender, symmetrical disease onset and autonomic dysfunction were identified as independent significant factors contributing to latency to first fall in PD. Both older age and female gender are well recognised risk factors for falls in the general population (Campbell *et al.*, 1989; Lord *et al.*, 1993; Tromp *et al.*, 1998) and have been identified as risk factors for falls in some, but not all reports in PD. (Bloem *et al.*, 2001; Ashburn *et al.*, 2001; Wielinski *et al.*, 2005) This study also suggests that falls occur more frequently in women and they tend to fall significantly earlier than men.

Falls in MSA

The clinical factors that were more common in fallers with MSA were similar to those found in PD, in particular axial symptoms and signs (early axial rigidity, speech disturbance and dysphagia) and early pyramidal tract signs. Less than 15% of cases in this study presented with prominent cerebellar symptoms (MSA-C) accounting for the

lack of association with cerebellar signs. Interestingly autonomic dysfunction, which was present in more than 50% of patients in the first two years of disease, was not significantly associated with the risk of falling. The clinical features which significantly and independently influenced latency to first fall were the same as PSP: age of disease onset and postural instability.

Fractures

More than one quarter of patients with PSP (28.6%) fractured at least one bone and the frequency of fractures was significantly higher than in the other diseases (Fisher's exact test: $p<0.02$). The clinical features that were more frequent in those with fractures and PSP were also different to other diseases. Whereas in PD, MSA and control populations women were more at risk of fracture (Nordell *et al.*, 2000) in PSP there was no association with female gender. In PSP only the absence of early bradykinesia, limb rigidity and pyramidal signs were significantly associated with fractures. When early bradykinesia, limb rigidity and pyramidal signs are present, patients maybe less likely to attempt to independently mobilise and therefore there maybe fewer opportunities of succumbing to the sequelae of poor balance and "motor recklessness" making fractures less frequent. Among the elderly in residential care facilities men fall more than women but fractures are more frequent in women. (Sadigh *et al.*, 2004) In contrast, men with PSP had a similar propensity to fracture bones perhaps reflecting the nature of falling associated with the disease and the frontal cognitive deficits in many patients. There was no difference between clinical phenotypes in gender distribution. A similar pattern was seen in patients with VP, who often had some associated cognitive impairment.

The proportion of hip fractures was higher in PD than in PSP and MSA. (Table 11.17) There were a significantly smaller proportion of fractures at the ankle and foot in MSA than in PD and PSP. Fractures of the trunk (ribs, vertebrae and sternum) were proportionally more in PSP and MSA than in PD. In contrast to previous reports (Wielinski *et al.*, 2005) rib and vertebral fractures were proportionally less than hip and proximal upper limb fractures in PSP. These studies have different methodology and the discrepancy may be explained if rib and vertebral fractures occur earlier in the disease,

and hip and arm fractures later. In other respects, the location of fractures was similar between disease groups.

Nordell and colleagues reported that in a residential population of 65 to 74 year olds fractures of the arms, and in particular the distal radius, were most common with fractures of the proximal femur or hip being half as common as radial fractures. (Nordell *et al.*, 2000) In all bradykinetic rigid syndromes diagnosed in this study this ratio was reversed, probably reflecting a common disturbance of postural reflexes. Bradykinesia prolongs reaction times following a postural challenge thereby compromising the protective responses, including outstretching of the arm, and thus limiting fractures of the forearm and wrist.

Conclusions

Falls and fractures are frequently associated with bradykinetic rigid syndromes. The disease burden associated with disturbances of posture, resulting in falls and factures appears to be highest in PSP. The significant difference between RS and PSP-P in latency to first fall supports a division of these clinical syndromes. While the mean latency to first fall is shorter in PSP-P than PD, the differences are not sufficiently discriminatory on their own to be helpful in the diagnosis of PSP-P. Patients with PSP, PD, MSA, DLB and VP should be assessed for the independent risk factors identified in this study.

The presence of early supranuclear gaze palsy, cognitive change and a lack of response to levodopa predict early falls and bone fractures in PSP. Patients with RS, and those with other bradykinetic rigid syndromes with symmetrical disease onset, postural instability, autonomic dysfunction and cognitive disturbance, and in particular women, may benefit from early assessment for osteoporosis, treatment of decreased bone mineral density and physiotherapy intervention to limit falling related morbidity. (Sato *et al.*, 2001)

11.2.3. VH in pathologically diagnosed bradykinetic rigid syndromes

Background

VH are considered rare in bradykinetic rigid syndromes other than PD, and have not been systematically studied in pathologically diagnosed series that include these patients. There are, however, some clinical reports of VH in MSA, PSP and CBD. (Lees and Bannister, 1981; Aarsland *et al.*, 2001; Shimo *et al.*, 2001; Nagaoka *et al.*, 2004) In these conditions LB do not usually occur in the frontal or temporal cortices, implying that when present VH are not due to Lewy body accumulation in the cortex. This distinction may be helpful when clinically diagnosing bradykinetic rigid syndromes, particularly when attempting to distinguish idiopathic PD from non-Lewy body Parkinsonism.

Aims

The aims of this study were to evaluate the relationship between pathological diagnosis and VH, specifically to determine if the presence of VH is helpful in distinguishing PSP-P from PD, and to quantify the temporal evolution of VH in each disease and analyse the relationship between VH and other clinical factors including medications.

Patients and methods

Patients

A retrospective analysis was performed using the clinical files of 788 patients with a lifetime diagnosis of a bradykinetic rigid syndrome or parkinsonism with a pathologically confirmed diagnosis (PD, n = 473; DLB (where cognitive dysfunction was recorded within the first year of disease onset), n = 44; PSP, n = 127; MSA, n = 91; VP, n = 27; AD, n = 9; CBD, n = 9; PEP, n=8) archived at the QSBB. All cases met the currently accepted pathological criteria for the diagnosis of these conditions. (Kosaka, 1990; Mirra *et al.*, 1991; McKeith *et al.*, 1996; Litvan *et al.*, 1996b; Gilman *et al.*, 1999; Lantos, 2000) A number of cases could not be included in the analysis because of insufficient clinical data (28 PD, 7 PSP, 5 MSA, 2 AD, 2 VP). PD and DLB groups were combined (Lewy body Parkinsonism, LBP) for analysis because of the similar

pathological substrate and arbitrary clinical definition which relies, in part, on the presence of VH in early disease to differentiate them.

Data Collection

A systematic chart review was performed as described in chapter 6. VH were recorded as present if they were specifically recorded, including formed VH and Fenelon's minor form (presence, passage or illusion). (Fenelon *et al.*, 2000) Time from disease onset to first VH (latency) was also recorded. Patients with isolated auditory or tactile hallucinations were not included. Visual acuity and field loss were not recorded in most patient files. Complete medication history was obtained, including latency to initiation and maximum dose for all medications except apomorphine and time to maximum dose for levodopa. The rate of levodopa increase after initiation, designated 'levodopa gradient' (LG), was calculated: LG = maximum dose/time to reach maximum dose. Dopamine agonists (DA) were sub-divided into ergot and non-ergot groups in a further attempt to establish if a difference exists between their hallucinogenic potential. (Brunt *et al.*, 2002) Calculation of a daily levodopa equivalent unit (LEU) dose was based on theoretical equivalence to levodopa (Evans *et al.*, 2004) as follows: bromocriptine (mg) x 10, cabergoline (mg) x 67, pergolide (mg) x 100.

Statistics analysis

The report of any VH from the time of disease onset until death was the primary outcome measure. The ability to predict LBP (PD and DLB) by the presence of VH was examined by calculating sensitivity, specificity, PPV and NPV against all the other diseases where Lewy bodies are not considered the pathological hallmark. Clinical characteristics in different diseases were compared. We investigated the associations between falls, bradykinesia, rigidity, tremor, cognitive dysfunction, speech disturbance, dysphagia, dystonia, dyskinesias, autonomic dysfunction, gender, symmetry of clinical features and age at disease onset with time to hallucinations in LBP. Univariable analyses using χ^2 for categorical and two-tailed *t* test for continuous variables were applied. Cox survival analysis was performed for LBP with latency to first VH and clinical factors present around disease onset (within the first two years) and gender as

categorical covariates and age at onset as a continuous variable. The influence of medications on VH was investigated using Spearman's correlation calculations for latency to medication initiation as a percentage of total disease duration versus latency to first VH as a percentage of disease duration in an attempt to correct for variability in disease length and severity. The maximum levodopa dose and LG were also compared to latency to first VH. The time from VH to death, expressed both as absolute time and percentage of disease duration, was compared amongst PD hallucinators.

Results

Amongst the 744 cases the incidence of VH was 36.6%. In PD, VH were reported in 221 (49.7%) patients and in DLB in 32 (72.7%). (Table 11.18) Eight patients (6.8%) with PSP experienced VH. In four, these were caused by dopaminergic medication and ceased on withdrawal of the offending medication and in a fifth there was a long history of alcohol dependence and post traumatic stress disorder. These patients were included in further analysis. Eight patients (9%) with MSA experienced VH and in three they occurred only after starting dopaminergic medications and did not persist after medication withdrawal. VH were reported in only one patient with VP (4%) and one with PEP (13%), but in none with AD or CBD. Table 11.18 shows the list of anti-Parkinsonian medications used in the different disease groups.

The specificity of VH for LBP was 92.9% and the PPV was 93.4% (95% CI 89.7 to 95.8), but sensitivity was 51.7% and NPV was 50.1% (95%CI 45.62 to 54.6). In LBP VH occurred a mean of 11 years after disease onset (PD mean 11.9 years, range 4.0-37.0, standard deviation(SD) 6.2; DLB mean 4.6, range 0.1-14.0, SD 3.5). There was no difference in disease duration between hallucinators and non-hallucinators. (Table 11.19). In PD hallucinators, VH occurred a mean of 53 months prior to death (standard deviation 31.7 months; interquartile range (IQR) 25%-75%, 26-73 months), which corresponded to VH onset at 70% of total disease duration (IQR 25%-75%, 60-84%). (Figure 11.9). Correspondingly, those who experienced hallucinations earlier died earlier.

	PD	DLB	PSP	MSA	CBD	VP	AD	PEP
Number	445	44	120	86	9	25	7	8
Mean age at onset (range)	60.4 (28.6-82.4)	66.7 (43.0-82.6)	65.7 (40.1-87.5)	56.4 (33.5-79)	66.2 (55.6-76.1)	70.5 (59.4-81.3)	68.1 (60.5-75.4)	26.9 (9.1-38.1)
Mean age at death (range)	76.2 (49.8-93.4)	74.9 (54.9-88.3)	73.7 (45.4-95.8)	64.4 (39.4-86.0)	73.0 (65.8-81.6)	82.6 (72.4-93.6)	80.2 (71.0-94.6)	80.3 (71-84.6
Disease duration (years) (range)	15.8 (1.4-37.0)	8.2 (3.7-15.8)	8.0 (1.2-23.8)	8.0 (2.4-16.5)	6.8 (3.6-10.2)	12.1 (2.6-26.2)	8.3 (1.3-17.0)	53.5 (41.4-72.8)
Women (%)	169 (38)	9 (20)	45 (37)	49 (55)	6 (33)	11 (44)	2 (29)	4 (50)
Hallucinators (%)	221 (49.7)	32 (72.7)	8 (6.8)	8 (9)	0	1 (4)	0	1 (13)
Latency to VH (years) (range)	11.9 (0.9-34.0)	4.6 (0.1-14.0)	4.5 (2.0-11.0)	6.9 (4.0-11.0)		10.5		28
Medications								
Levodopa (%)	99.8	93	76	86	88	92	82	75
Mean maximum dose, mg/day (range)	935 (125-4400)	683 (250-1500)	667 (125-1500)	973 (375-3000)	833 (440-1125)	684 (250-1250)	647 (375-825)	759 (500-1375)
Ergot DA (%)	50	25	17	45	22	24	22	38
Not-ergot DA (%)	10	7	7	7	11	0	0	0
Selegiline (%)	72	41	24	50	33	44	33	13
Amantadine (%)	29	25	28	26	22	8	0	38
Anticholinergic (%)	55	27	22	42	11	28	22	63

Table 11.18 Clinical features according to pathological diagnosis

	Hallucinators	**Non-hallucinators**	***p value****
Patients			
Number	253	236	
Women (%)	82 (37)	95 (40)	
Age of onset	60.2 (30-82.6)	61.7 (28.6-82.3)	0.11
Disease duration (years)	14.6 (4.0-37.0)	15.6 (1.4-41)	0.11
Levodopa			
Number (%)	250 (99)	233 (99)	0.71
Latency (months)	34 (1-216)	38 (1-217)	0.32
Mean dose (mg)	944 (125-4400)	895 (250-2750)	0.32
Levodopa gradient	12.0 (0-25)	10.4 (0-18)	0.23
Ergot DA			
Number (%)	135 (53)	100 (42)	0.015
Latency (months)	117 (10-401)	122 (26-390)	0.58
Mean dose (LEU)	579 (15-1400)	622 (15-1300)	0.62
Non-ergot DA			
Number (%)	28 (11)	16 (7)	0.10
Latency (months)	166 (56-343)	185 (41-432)	0.47
Selegiline			
Number (%)	185 (73)	152 (64)	0.032
Latency (months)	98 (6-324)	104 (7-341)	0.41
Mean dose (mg)	8.9 (5-20)	8.8 (5-15)	0.74
Amantadine			
Number (%)	90 (36)	48 (20)	<0.001
Latency (months)	94 (2-330)	87 (2-264)	0.65
Mean dose (mg)	220 (100-400)	184 (100-300)	0.005
Anticholinergic			
Number (%)	136 (54)	121 (51)	0.58
Latency (months)	57 (2-396)	61 (2-400)	0.62

Table 11.19 Medication use in patients with LB pathology (mean, (range), *χ^2 or Student's t-test)

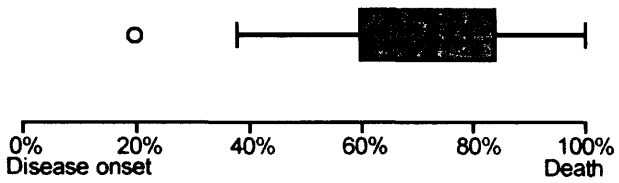


Figure 11.9 Time of VH onset as a function of total disease duration in PD, median, interquartile range, outlier

LBP hallucinators were significantly more likely to be taking ergot derived DA, selegiline and amantadine (table 11.19). There was no association between anticholinergic medication and VH. Levodopa was used equally by hallucinators and non-hallucinators and there was no difference between these groups in maximum dose or time to reach maximum dose. In PSP, MSA, CBD, PEP and VP there was no difference in medication use between hallucinators and non-hallucinators.

In patients with LBP, univariable analysis showed an association between VH and cognitive dysfunction (χ^2, p=0.002), falls (p=0.005), postural instability (p=0.007), speech disturbance (p<0.0001), dysphagia (p=0.015), and autonomic dysfunction (p=0.041). Latency to first VH as a percentage of disease duration only weakly correlated with time to initiation of ergot dopamine agonists (Spearman's rho 0.24, p=0.006) and selegiline (0·22, p=0.005) expressed as percentages of disease duration, but not time to maximum levodopa dose (0.11, p=0.15), amantadine (0.17, p=0.14), or anticholinergic drugs (0.14, p=0.11; table 10.20). The levodopa gradient and the maximum dose of levodopa were not correlated with latency to onset of VH. The assumptions for covariates used in multivariate analyses were found to be correct. The survival curves showed several early clinical factors that were independent predictors of onset of VH in patients with LBP (table 11.21).

Onset of hallucinations and time to:	Spearman's correlation	*p*
Maximum levodopa dose	0.11	0.146
Initiation of ergot dopamine agonist	0.24	0.006
Initiation of selegiline	0.22	0.005
Initiation of amantadine	0.17	0.139
Initiation of anticholinergic medication	0.14	0.114

Table 11.20 Spearman's correlation coefficient between time to onset of VH in LBP and time to initiation of medication expressed as a percentage of disease duration

Early clinical features	Hazard Ratio	95% CI	*p*
Cognitive dysfunction	5.62	3.37-9.35	<0.001
Autonomic dysfunction	3.13	1.77-5.52	<0.001
Axial rigidity	2.22	1.26-3.85	0.006
Age of onset	1.05	1.03-1.07	<0.001
Asymmetric onset	0.60	0.40-0.91	0.015

Table 11.21 Factors predictive of VH in LBP: multiple Cox regression analysis

Discussion

VH are common in PD and very uncommon in unmedicated PSP and MSA. VH are a frequent clinical accompaniment of LB pathology and are very infrequently seen in PSP and other bradykinetic rigid syndromes. The presence of VH predicted LBP with 93% accuracy. VH occurred in 49.7% (221) of patients with PD and in nearly all cases VH occurred in the second half of the disease course. VH only weakly correlated with use of selegiline and ergot DA and not with levodopa use, amantadine or anticholinergic medications. They occurred earlier in patients with early cognitive dysfunction, prominent axial rigidity and autonomic dysfunction. Of the 15% of patients with LB pathology who experienced VH within 5 years of disease onset, most had a clinical syndrome consistent with DLB and the associated pathological features. Less than 4% of hallucinators with PD developed VH in the first 5 years.

In this study, the incidence of VH in PD was higher than previous cross sectional and longitudinal studies of shorter duration. (de Maindreville *et al.*, 2005) The

retrospective methodology, using case notes without screening specifically for VH, means that this figure is also likely to be an underestimate of the true incidence. The proportion of patients in this study with non-LBP is higher than would be predicted by most community estimates of prevalence. (Schrag *et al.*, 1999) Predictive values of VH for LBP are dependent on the prevalence of the diseases in the test population and therefore the PPV is likely to be higher than we have reported because of the over representation of non-LBP in this clinicopathological series and limits the generalisation of these findings. (Maraganore *et al.*, 1999) Nonetheless these limitations applied equally to the different diseases, and should not influence the specificity of the results. We chose to record the first experience of VH in an attempt to clarify the clinical relevance of VH to the progression of disease and it was not possible to distinguish persistent VH from a single acute episode related to drug changes in many cases.

Once established, VH tend to be stable (de Maindreville *et al.*, 2005), probably reflecting encroachment of α-synuclein pathology into central visuoperceptual systems. (Diederich *et al.*, 2005) In the context of pathological progression in Parkinson's disease, this progression would correspond to Braak stage 4 or 5. (Braak *et al.*, 2003) Although the onset of VH does not suggest a poorer prognosis than if there were no VH, progression to the second half of the course of PD is suggested. (Figure 11.9)

The relationship between dopaminergic therapy and the onset of VH is complex. (de Maindreville *et al.*, 2005) The facilitating role of dopaminergic treatment is well established, but VH are not an invariable complication of treatment and rarely occur in other conditions where dopaminergic treatment is used such as hyperprolactinaemic infertility and Ekbom's syndrome. We found that the total dose and frequency of exposure to levodopa was similar between our LBP and non-LBP groups (see table 11.18). In most published clinical studies in PD levodopa dose did not correlate with the frequency or severity of VH. (Fenelon *et al.*, 2000; de Maindreville *et al.*, 2005) This is further supported by our findings that total levodopa dose, rate of levodopa increase and maximum levodopa dose do not independently correlate with VH or the time to first hallucination in PD or DLB. Ergot DA, selegiline and anticholinergic medications were used more often in LBP than PSP, MSA and other non-LBP. Amongst the LBP hallucinators, DA, selegiline and amantadine were prescribed more frequently than in

non-hallucinators (see table 11.19) but the time to onset of VH did not correlate with exposure to the later and only weakly with ergot DA and selegiline when corrected for disease duration at the time of VH onset. While these medications may be more hallucinogenic than others, these results support the notion that the pathological substrate has a greater influence on the development of VH than the medications alone. (Holroyd *et al.*, 2001) Anticholinergic drugs are known to be a potent cause of hallucinations in patients without clinical evidence of neurodegenerative disease. (Perry and Perry, 1995) Extensive neocortical cholinergic deficits exist in DLB that are thought to contribute to VH and fluctuations of cognition, as well as to the sensitivity of these patients to anticholinergic and dopaminergic medications. (Perry and Perry, 1995) It is surprising that we have not found a correlation between time to VH onset and cholinergic medication use in LBP and may reflect patient selection. Because anticholinergic drugs have a mild effect on the motor features of PD and the tendency for cognitive dysfunction increases with disease progression, treating physicians are more likely to withdraw drugs or never prescribe them in patients at highest risk, which may explain the lack of correlation.

The association between VH and cognitive dysfunction and axial symptoms, including falls and dysphagia, is well established, (Levy *et al.*, 2000) and this combination may help to discriminate PSP-P from PD. Cognitive dysfunction was shown to have the largest influence on time to VH using Cox survival analysis (table 11.21). We found that autonomic dysfunction (see definition above) was also associated with VH and significantly influenced latency to first hallucination. Cognitive dysfunction, axial symptoms and autonomic dysfunction imply widespread LB pathology and are all common findings in DLB. (McKeith *et al.*, 1996) The risk of VH increased with increasing age of disease onset which may reflect the incidence of dementia in older patients and the superimposed effects of ageing. Ocular disorders are known to influence VH in PD (Diederich *et al.*, 2005) and are more likely to occur in older patients. (Teunisse *et al.*, 1996) REM sleep behaviour disorder and altered sleep-wake cycles have also been linked with VH in PD (Pappert *et al.*, 1999) but were not assessed. VH were less likely to develop in those patients with asymmetrical disease and those with tremor or dyskinesias. These clinical features may reflect the limited extent of pathological

burden, or specific alterations in the dopaminergic or serotonergic systems, (Bibbiani *et al.*, 2001; Diederich *et al.*, 2005) that may reduce the risk of developing VH.

Conclusions

In patients with Parkinsonism, VH are highly specific for LBP and, when they occur in PD, indicate that, in that individual, more than half of the disease course has passed. The emergence of VH is not caused directly by dopaminergic medications but is influenced by the interaction of these medications with progressive α-synuclein pathological involvement of visuoperceptual systems. These findings need to be confirmed with a prospective cohort of clinically, and then pathologically diagnosed group of patients.

VH are a useful clinical sign associated with underlying LB pathology in Parkinsonism, and should be specifically asked for in the clinic. Minor and major hallucinations, as defined by the QSVHI, are very rarely reported in clinically and pathologically diagnosed PSP. In patients with unclassifiable Parkinsonism the presence of VH is highly suggestive of PD or DLB, rather than PSP-P or MSA. If however, VH precede the onset of Parkinsonism or completely disappear on withdrawal of hallucinogenic medications, non-LB pathology remains possible.

7.12. Sample size calculations using clinical phenotypes.

The effect of separating the clinical phenotypes of PSP on the sample size needed to detect a treatment effect in prospectively studied patients was examined. Sample sizes required to detect the effect of a proposed disease-modifying treatment were estimated for all clinically diagnosed patients, for those with the correct clinical diagnosis of PSP and those clinically categorised as RS. For a treatment effect equivalent to a 20% (14 months) improvement in longevity, fewer PSP subjects would be required in each treatment arm when recruiting only those patients who fit the RS clinical phenotype (252 cf. 284). Power calculations based on RS and PSP-P clinical subgroups confirm that this designation has the potential to improve the ability of clinical trials to evaluate the efficacy of a disease modifying treatment in PSP.

Introduction

Progressive supranuclear palsy (PSP) is diagnosed by identifying a symmetric bradykinetic rigid syndrome with early falls, a supranuclear gaze palsy (SNGP) and axial rigidity. (Steele *et al.*, 1964) The definitive diagnosis of PSP can only be made pathologically, as the accuracy in antemortem diagnosis is likely to be less than 90%. (Litvan *et al.*, 1996a; Osaki *et al.*, 2004) In the patients used for analysis in chapter 6.1 the mean disease duration in RS was 5.9 years and in PSP-P was 9.1 years, compared to all PSP cases where disease duration was 7.0 years. The difference in disease duration between each clinically diagnosed group may be helpful in trial design, by improving homogeneity in treatment groups.

Aims

The aim of this study was to determine the effect of identifying these phenotypes for clinical trials where disease duration is the primary outcome measure.

Methods

A retrospective analysis was performed using the clinical files of 758 patients archived at the QSBB, and included PD/DLB, n = 490; PSP, n = 127; MSA, n = 90; CVD, n = 25; AD, n = 9; CBD, n = 9 and PEP, n=8. All patients clinically diagnosed as

PSP and all pathologically diagnosed as PSP were included and separated according to the clinical phenotypes (RS and PSP-P).

Sample size requirements were estimated for number of events (deaths) needed to establish a 20% treatment effect on disease duration. The following standard formula was applied to estimate sample sizes in patients clinically diagnosed with PSP (Machin *et al.*, 1997) (including path proven PSP and other pathological diseases), patients correctly diagnosed clinically as PSP (all pathologically proven) and patients correctly diagnosed with PSP and RS clinical phenotype:

$$\textit{Number of events needed (E)} = 4(u + v)^2/\log (M_1/M_2)^2$$

where u = 1.28 (the one sided percentage point of the normal distribution corresponding to 100%- the power) to provide 90% power and v = 1.96 (the percentage point of the normal distribution corresponding to the two-sided significance level) to test at the 5% level; M_1 is median disease duration following final diagnosis in treatment group and M_2 is median disease duration following diagnosis in placebo group and therefore M_1/M_2=HR. Numbers needed for different trial durations were calculated according to the following: $E=(M_1+M_2/2)x$(trial durations). All calculations were performed based on the requirement that a trial should have 90% power to detect the specified treatment effect when a 2-sided 5% level of significance is used.

Results

Of the 758 cases reviewed, 114 had been clinically diagnosed with PSP. This diagnosis was confirmed in 93 (82%), and incorrect in 21 (18%, 12 (11%) with PD, 7 (6%) MSA and 2 (2%) CBD). The median time to final clinical diagnosis was lowest in RS (table 12.1). In the clinically diagnosed cases the median disease duration was 6.5 years (range 1.2-16.5; SD, 3.1 years). Eighty six of these patients were categorised as RS according to clinical features (median disease duration of 6.0 years, 1.2-13.5; SD, 2.4), including 76 with pathological PSP (89%), seven with pathological PD (8%), one with MSA (1%) and two with CBD (2%). The mean duration of disease was not significantly

different between correctly and incorrectly diagnosed patients categorised RS (6.4 *vs.* 6.7 *t-test p=0.76*).

In pathologically diagnosed patients the disease duration SD of RS was substantially lower than the PSP group as a whole. The sample size needed if only RS cases were to be included was around 25% less than if all PSP patients were included.

The results of the power calculations can be interpreted as follows: to have 90% power to detect the effect of a drug or intervention with an anticipated ability to increase disease duration by 20% (14 months) in all clinically diagnosed PSP patients, in a clinical trial with a 3 year follow-up period, 567 patients would be needed in total (table 12.2). To have the same power in a trial lasting 3 years in all clinically diagnosed PSP patients with only a RS phenotype, 504 patients would be needed in total.

		Median time to final diagnosis (range) *yrs*	Median disease duration (range) *yrs*	SD
Clinical diagnosis of PSP				
All	n=114	3.2 (0.4-14.5)	6.5 (1.2-16.5)	3.1
Correct diagnosis	n=93	3.1 (0.4-14.5)	6.4 (1.6-16.5)	3.1
RS phenotype only	n=86	2.9 (0.5-8.5)	6.0 (1.2-13.5)	2.4
Pathological diagnosis of PSP				
All	n=127	2.8 (0.3-14.5)	7.4 (1.2-23.8)	4.7
RS	n=86	2.8 (0.5-12.4)	6.1 (1.2-14.5)	2.5

Table 12.1 Homogeneity in clinical phenotypes of PSP

	Number of events needed	Trial duration (*yrs*) 2	3	4	5	6
Clinical diagnosis of PSP						
All	436	850	567	425	340	283
Correct diagnosis	436	850	567	425	340	283
RS phenotype only	403	756	504	378	302	252
Pathological diagnosis of PSP						
All	781	1134	756	567	453	378
RS	436	850	567	425	340	283

Table 12.2 Effects of clinical phenotype on sample size in clinically diagnosed PSP

Discussion

Power calculations based on RS and PSP-P clinical subgroups confirm that this designation has the potential to improve the ability of clinical trials to evaluate the efficacy of a disease modifying treatment in PSP.

The few therapeutic trials in PSP have failed to demonstrate consistent clinical benefits or disease modification. (Ghika *et al.*, 1991; Kompoliti *et al.*, 1998; Litvan *et al.*, 2001; Burn and Warren, 2005) With the development of several agents that have the potential to modify the natural history and even the pathophysiology of PSP (Burn and Warren, 2005), the scene is set for the development of appropriate research strategies to test these agents. However, in the absence of validated, sensitive clinical tools designed specifically for PSP, future trials for symptomatic and putative neuroprotective interventions risk type II error. Measures of functional status and quality of life in PSP have been proposed but have not been assessed prospectively. (Goetz *et al.*, 2003; Schrag *et al.*, 2005) In the absence of reliable markers of disease progression one strategy for testing disease modifying agents is survival analysis of treatment versus placebo. The sample sizes needed to perform this type of trial have been estimated using data from pathologically confirmed cases.

The shorter disease duration in RS compared to PSP-P reduces the sample size to detect a significant difference in survival. One of the other important factors in determining sample size is the heterogeneity (SD) of the studied disease group. This variability arises as a result of natural differences between individuals or as a result of pathophysiological or anatomical differences in disease. The more homogeneous the study population, the easier it is to detect a small treatment effect. By separating RS and PSP-P the homogeneity, and therefore the power of clinical trials using any marker of disease progression is likely to be enhanced, and it is recommended that these considerations are included in future trial designs.

The prevalence of PSP is around 5 per 100,000 (Schrag *et al.*, 1999; Nath *et al.*, 2001), and compared to PD it is relatively rare, suggesting a small pool of available potential participants for any clinical trial. Sample size calculations indicate that by identifying and recruiting RS patients, 504 patients would need to be included in a

clinical trial to detect significant improvement in longevity, compared to 567 for clinically diagnosed PSP. The resources needed for this type of trial would be substantial and argue for the development of some validated surrogate marker for disease progression.

Several limitations are acknowledged in these findings. Firstly archived brain bank cases are not entirely representative of community based cases. (Maraganore *et al.*, 1999) Ascertainment bias from autopsy series favour patients with shorter disease durations, however the long recruitment period and consistency with other clinical reports suggest that this effect is likely to be small and outweighed by the strength of pathological confirmation. Finally the clinical designation was based on principal components factor analysis, and has not been confirmed prospectively.

Time to death is a reliable end point for the design of trials in PSP, and the numbers needed to power the trials are less if patients with only RS are included. Even if techniques for confirming the presence of PSP-tau pathology in life are developed, or surrogate markers of disease progression are validated, the stratification of RS and PSP-P will increase the homogeneity of clinical trials and therefore limit the likelihood of type II error.

Conclusions

Conducting a large multi-centre trial using time to death as a reliable end point, might be practical if patients with RS are identified. Such a trial would require 504 patients and could be complete within 5 years of recruitment.

In 1963 Richardson described a distinctive clinical syndrome that was found to be associated with subcortical gliosis and pathological tau accumulation in a number of subcortical regions. Richardson called it progressive supranuclear palsy and it is now recognised as the second most common cause of neurodegenerative Parkinsonism.

The pathological characteristics of this distinct clinical syndrome have broadened with technological advances in immunohistochemistry, electron microscopy and protein analysis. PSP-tau pathology shares much in common with other tauopathies, and in particular CBD, PEP and PDC-Guam, but can be defined by relatively severe tau pathology in subcortical nuclei and the presence of tufted astrocytes. Pathological differentiation, however, in individual cases is sometimes difficult. Regional pathological heterogeneity has previously been identified but the lack of apparent clinical correlations has confounded efforts to improve clinical and pathological diagnostic criteria.

It has become evident that the pathological diagnosis of PSP can be made in patients who do not develop many of the clinical features described by Richardson. These patients have previously been referred to in the literature as "atypical" PSP.

This thesis has explored the clinical and pathological heterogeneity in PSP and two other clinical syndromes have been identified that allow for a more refined classification of this prototypic primary tauopathy. The continuum of clinical and pathological features in PSP and the overlap with CBD, PEP and PDC Guam, makes absolute distinction between these clinicopathological entities impossible.

Richardson's syndrome, identified by onset of falls, supranuclear eye movement abnormalities and cognitive dysfunction within the first two years of disease, is distinct from other forms of Parkinsonism. It is recognised with high accuracy in movement disorders clinics and is characterised by relentless deterioration to death within six or seven years. The pathological hallmarks of RS are neuronal and glial tau pathology in specific nuclei in the brainstem, cerebellum and, contrary to original reports, in some

areas of the cerebral cortex. Tau accumulation in the pontine base is made of predominantly 4R-tau.

The clinical syndrome of PSP-Parkinsonism differs from RS. Clinical features within the first two years of disease have more in common with PD than RS, often with asymmetric onset of tremor and moderate response to levodopa. The supranuclear gaze palsy, cognitive dysfunction and falls occur later, if at all, and the mean disease duration to death is about 10 years. Pathological differentiation of PSP-P from RS is not straight forward, and the substantial pathological overlap between these syndromes implies a common ætiopathologenetic substrate. While in both syndromes the STN and SN are most severely affected, in PSP-P the distribution of pathology outside of these structures is more limited. A difference in pathological tau biochemistry hints at some modification of the pathological process involved in PSP-P compared to RS. Trends in the odds ratios for the PSP-susceptibility tau haplotype suggest that genetic factors could be responsible for the differences, but tau mutations appear not to be.

Pure akinesia with gait freezing is a further distinct clinical entity and the absence of rigidity and other signs of RS and PD distinguish this syndrome. It appears to rarely be associated with Lewy body pathology and may be confused with vascular Parkinsonism. Patients with PSP-PAGF have longer disease duration than RS. In general the pathological-tau severity is less and is more limited in its extent than in PSP-PAGF than in RS.

The accuracy of applying operational diagnostic criteria in movement disorders clinics is skewed by differences in the prevalence of different diseases. The specificity of the criteria for diagnosing PD is not sufficiently high to reliably exclude PSP-P. Identifying the presence of visual hallucinations increases the specificity for PD, and is an important exclusion criterion for the diagnosis PSP. In both RS and PSP-P the mean time to first fall is significantly less than PD, emphasising the importance of this clinical feature in the clinical prediction of underlying PSP-tau pathology. However, the specificity of falls before 6 years is not sufficiently high to separate PSP-P from PD. Olfaction is not always normal in PSP, but is much more likely to be preserved than in PD. A severely abnormal result on UPSIT testing is suggestive of underlying Lewy body pathology, increases the specificity for PD and makes PSP unlikely. Electrophysiological

testing of the auditory startle response is usually abnormal in established RS and PSP-P, and the degree of abnormality probably reflects the extent of pathological involvement in the midbrain and pons. Abnormalities in both ASR and ABR suggest RS rather than PD.

The extension of clinical observations in these studies challenges the classic view of PSP and, according to Charcot's anatomoclinical approach, argues for a nosological separation of RS, PSP-P and PAGF. All three conditions are clearly linked by the presence of characteristic pathological lesions in STN, SN and GP, but are separated by the extent and distribution of that pathology, their natural history and biochemical profile.

The clinical differences between RS, PSP-P and PAGF are sufficient to warrant separation from 'PSP', in the same way that PEP and PDC Guam are considered separately. These differences should be acknowledged and a modified understanding of what, exactly, constitutes PSP may improve efforts to discover aetiological factors and disease modifying therapies. Problems with the nosology of PSP are not unusual, and the same issues confound the understanding of PD, AD and MND. (Calne, 2005) In these conditions conventional clinical and anatomoclinical definitions of disease are being adapted to incorporate advances in molecular biology and aetiopathogenesis. A sub-classification of the extent and distribution of PSP-tau pathology would also provide a plausible framework for considering further clinicopathological correlations. The pathological overlap with PDC Guam, PEP and pallidonigro-luysian atrophy needs further clarification and, if a definitive classification is to be achieved, future studies need to critically evaluate the pathological differences between these diseases and RS, PSP-P and PAGF.

A proportion of patients with unclassifiable Parkinsonism will go on to develop PSP-P, and a small proportion of patients with clinically diagnosed PD will prove to have PSP-tau as the underlying pathology. These patients can be identified by the absence of drug induced dyskinesias, autonomic failure and visual hallucinations, the presence of falls within 6 years of disease onset, UPSIT scores above the 12th percentile for gender and age, and abnormalities in ASR. Even if the diagnosis of PSP is made late in the disease these patients should be stratified separately in clinical trials because the underlying pathology is likely to be less extensive and less severe and the clinical features and prognosis are different to RS. Patients whose clinical features fit the profile

of PSP-P, and where PAGF, corticobasal syndrome or progressive non-fluent aphasia is diagnosed, an accurate ante-mortem diagnosis of PSP-tau pathology may be best served by the development of a biomarker for the presence of tau pathology. An understanding of the genetic and environmental factors that contribute to clinical and pathological heterogeneity in PSP is likely to shed further light on the mechanisms that lead to tau pathology.

The genetics the easel,
and proteins the paint;
History the brush strokes,
first symptoms health's feint.

With science and series,
a picture is made;
After death the answer?
Charcot's method to sate.

This picture we see,
is just words in a book;
For disease, this is not,
ask the patient, then look.

D Williams 2006

Peer review publications related to this thesis

Williams DR.
Tauopathies: classification and clinical update on neurodegenerative diseases associated with microtubule associated protein tau
Internal Medical Journal 2006;36(10):652-60

Williams DR, Lees AJ.
Falls and fractures: predictors and prognosis in pathologically diagnosed bradykinetic rigid syndromes
JNNP 2006;77(4):468-73

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Lancet Neurology, 2005;4(10):605-610

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Williams DR, de Silva R, Paviour D, Pittman A, Watt H, Kilford L, Holton J, Revesz T, Lees AJ.
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Movement Disorders – in press

Williams DR, Holton J, Strand C, Pittman A, de Silva R, Lees AJ, Revesz T
Grading of brain pathology in progressive supranuclear palsy - *submitted*

Williams DR, Holton J, Strand C, Revesz T, Lees AJ
Pure akinesia with gait freezing – natural history and pathological findings - *submitted*

Williams DR, Steele JC, Wherrett JR, Lees AJ
J Clifford Richardson and fifty years of progressive supranuclear palsy - *submitted*

Doyle LM, Williams DR, Lees AJ, Brown P
Loss of acoustic blink and startle reflex in progressive supranuclear palsy - *submitted*

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I am lucky to have been accompanied along this journey by Libby (PhD of funk) and joined by Mia (PhD of cuteness), who have kept it great fun.

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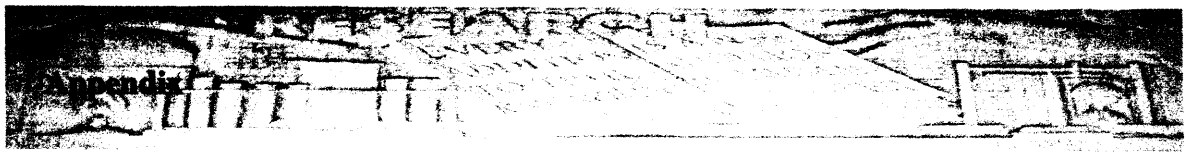


UKPDSBB Criteria for the diagnosis of PD (Gibb and Lees, 1988)

Step 1: Diagnosis of Parkinsonian syndrome

- Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions)

And at least one of the following:

- Muscular rigidity
- 4-6 Hz rest tremor
- Postural instability not caused by primary visual, vestibular or proprioceptive dysfunction

Step 2: Exclusion criteria for PD

- History of repeated strokes with stepwise progression of Parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language and praxis
- Babinski's sign
- Presence of cerebral tumour or communicating hydrocephalus on computed tomography
- Negative response to large dose of levodopa
- MPTP exposure

Step 3: Supportive prospective positive criteria for PD (three or more required for diagnosis of definite PD)

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset
- Excellent response to levodopa (70-100%)
- Severe levodopa induced chorea
- Levodopa response for 5 years or more
- Clinical course of 10 years or more

Consensus criteria for the diagnosis of multiple system atrophy (Gilman *et al.*, 1999)

Clinical domains, features and criteria used in the diagnosis of MSA. A feature (A) is a characteristic of the disease and a criterion (B) is a defining feature or composite of features required for diagnosis

I. Autonomic and urinary dysfunction
  A. Autonomic and urinary features
    1. Orthostatic hypotension (by 20 mmHg systolic or 10 mmHg diastolic)
    2. Urinary incontinence or incomplete bladder emptying
  B. Criterion for autonomic failure or urinary dysfunction in MSA
    Orthostatic fall in blood pressure (by 30 mmHg systolic or 15 mmHg diastolic) or urinary incontinence (persistent, involuntary partial or total bladder emptying, accompanied by erectile dysfunction in men) or both
II. Parkinsonism
  A. Parkinsonian features
    1. Bradykinesia (slowness of voluntary movement with progressive reduction in speed and amplitude during repetitive actions)
    2. Rigidity
    3. Postural instability (not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction)
    4. Tremor (postural, resting or both)
  B. Criterion for parkinsonism in MSA
    Bradykinesia plus at least one of items 2 to 4
III. Cerebellar dysfunction
  A. Cerebellar features
    1. Gait ataxia (wide based stance with steps of irregular length and direction)
    2. Ataxic dysarthria
    3. Limb ataxia
    4. Sustained gaze-evoked nystagmus
  B. Criterion for cerebellar dysfunction in MSA
    Gait ataxia plus at least one of items 2 to 4
IV. Corticospinal tract dysfunction
  A. Corticospinal tract features
    1. Extensor plantar responses with hyperreflexia
  B. Corticospinal tract dysfunction in MSA: no corticospinal tract features are used in defining the diagnosis of MSA

Exclusion criteria for the diagnosis of MSA

I. History
  Symptomatic onset under 30 years of age
  Family history of a similar disorder
  Systemic diseases or other identifiable causes for features listed in Table 1
  Hallucinations unrelated to medication
II. Physical examination
  DSM criteria for dementia
  Prominent slowing of vertical saccades or vertical supranuclear gaze palsy*
  Evidence of focal cortical dysfunction such as aphasia, alien limb syndrome, and parietal dysfunction
III. Laboratory investigation
  Metabolic, molecular genetic and imaging evidence of an alternative cause of features listed in Table 1

*In practice, MSA is most frequently confused with Parkinson's disease or progressive supranuclear palsy (PSP) [14]. Mild limitation of upward gaze alone is nonspecific, whereas a prominent (>50%) limitation of upward gaze or any limitation of downward gaze suggests PSP [13]. Before the onset of vertical gaze limitation, a clinically obvious slowing of voluntary vertical saccades is usually easily detectable in PSP and assists in the early differentiation of these two disorders [13].

Diagnostic categories of MSA. The features and criteria for each clinical domain are shown in Table 1

I. Possible MSA: one criterion plus two features from separate other domains. When the criterion is parkinsonism, a poor levodopa response qualifies as one feature (hence only one additional feature is required).
II. Probable MSA: criterion for autonomic failure/urinary dysfunction plus poorly levodopa responsive parkinsonism or cerebellar dysfunction.
III. Definite MSA: pathologically confirmed by the presence of a high density of glial cytoplasmic inclusions in association with a combination of degenerative changes in the nigrostriatal and olivopontocerebellar pathways.

Possible criteria for the clinical diagnosis of vascular Parkinsonism (Zijlmans *et al.*, 2004a)

A. *Parkinsonism:*

- bradykinesia
- and at least one of the following:
    - rest tremor,
    - muscular rigidity,
    - postural instability not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction

B. Cerebrovascular disease:

- defined by evidence of relevant cerebrovascular disease by brain imaging (CT or MRI)
- *or* the presence of focal signs or symptoms that are consistent with stroke

C. A relationship between the above two disorders. In practice:

- An acute or delayed progressive onset with infarcts in or near areas that can increase the basal ganglia motor output (GPe or substantia nigra pars compacta) or decrease the thalamocortical drive directly (VL of the thalamus, large frontal lobe infarct). The Parkinsonism at onset consists of a contralateral bradykinetic rigid syndrome or shuffling gait, within 1 year after a stroke (VPa).
- An insidious onset of Parkinsonism with extensive subcortical white matter lesions, bilateral symptoms at onset, and the presence of early shuffling gait or early cognitive dysfunction (VPi).

Exclusion criteria for VP:

- History of repeated head injury,
- History of definite encephalitis
- Neuroleptic treatment at onset of symptoms
- Presence of cerebral tumor or communicating hydrocephalus on CT or MRI scan
- Other alternative explanation for Parkinsonism.

Queen Square Visual Hallucination Inventory

MMSE: **UPDRS II (on):** **UPDRS III :**

H&Y: **1** **1.5** **2** **2.5** **3** **4** **5**

Psychiatric history:_____

Ocular pathology: □ **None** □ **Cataracts** □ **Retinal disease** □ **Glaucoma** □ **Spectacles**

A "Have you had any unusual visual experiences or hallucinations in the past 3 months?" **YES** **NO**

If YES "In relation to these experiences in the last 3 months....." →GO TO B
If NO "Have you had any unusual visual experiences more than 3 months ago?"

If YES "In relation to these experiences....." →GO TO B
If NO →GO TO B anyway to confirm absence of hallucinations illusions

B ***Minor hallucinations/illusions:***

"Have you had the vivid sensation of the presence of somebody in the room with you, when in fact there was no one there?"

"Have you experienced a brief vision of movement past you, of perhaps an animal or person, when in fact there was nothing there? → If yes, also complete page 2

"Have you looked at something and it appeared as something else for a time?"
"For example spots in the wall appearing as insects, or faces in patterns on fabrics or the carpet?"

C ***Formed visual hallucinations:***

"Have you had visions of people, animals or objects that were in fact not there?"

"Did you hear these people/animals/objects make any noise?"

D ***Auditory hallucinations:***

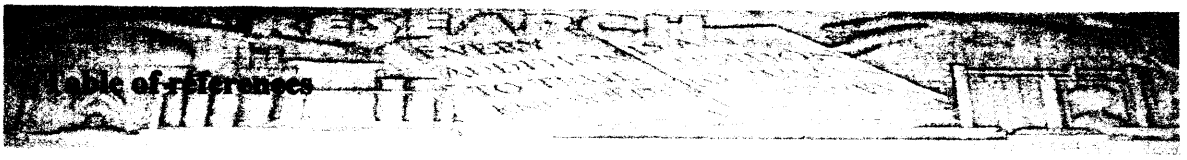
"Have you heard sounds of people talking, music or other noises when in fact there was no sound?"

E ***Details:***

"When did these unusual experiences start?" MM/YY "...and finish?" MM/YY

"Were they related **ONLY** to medications?"

Were the visual experiences related to delirium (as defined by DSM-IV)?



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